Estd. : July 1976

MANUAL for the LABORATORY TECHNICIAN at the PRIMARY HEALTH CENTRE

for the use by the students of IPH&H for

DIPLOMA IN MEDICAL LABORATORY TECHNOLOG

1992-94

Reprinted by:

INSTITUTE OF PUBLIC HEALTH & HYGIENE

95, Krishan Nagar [Street No.5] P.O. Safdarjung Enclave, New Delhi - 110-029

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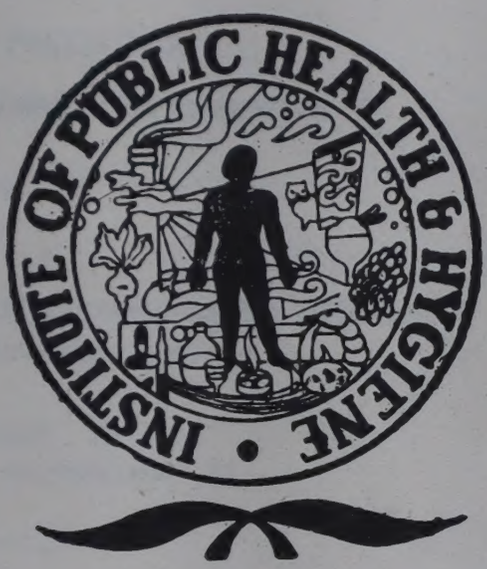

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Contents

How to Use the Manual

This Manual is intended for your use as a Laboratory Technician attached to the Primary Health Centre. The Manual covers only such procedures as you are expected to carry out at the PHC with the limited equipment at your disposal. Your job responsibilities are given in the preliminary pages of this Manual.

The Manual is divided into two main parts:

Part I : General Laboratory Procedures
and
Part II : Special Laboratory Procedures

Part I consists of the following chapters:

Chapter 1 : Maintenance of cleanliness of the laboratory
Chapter 2 : The microscope
Chapter 3 : Sterilization
Chapter 4 : Disposal of specimens and infected materials
Chapter 5 : Records and reports
Chapter 6 : Safety measures in the laboratory

A clean and neat laboratory provides a congenial atmosphere for working in and can help you to work in a more efficient manner.

For accurate results glassware should be scrupulously clean, and for certain procedures, e.g. collection of blood samples, sterilization of the equipment is essential to protect the patient from cross infection.

For correct interpretation of the results of microscopic examination, it is necessary for the microscope lenses to be kept clean and free from fungus, and for the microscope to be handled with care.

After carrying out any laboratory investigations the specimens should be safely disposed of and the containers cleaned and disinfected or properly disposed of.

Care in labelling of specimens and in recording the results is essential to avoid mixing up specimens with consequent mixing up of results.

All these procedures have been dealt with in Part I of this Manual.

How to avoid accidents and what to do in case of accidents in the laboratory are dealt with in Chapter 6.

A suggested list of simple drugs and dressings which every laboratory should keep handy for first aid is given at Appendix 6.1.

Appendix 1.1 includes a suggested list of materials, viz. furniture, equipment, glassware, chemicals, and miscellaneous items such as linen and cleaning materials, to be supplied to the PHC laboratory. When certain items are not provided, you should try to improvise substitutes. For instance, a simple test tube rack can be made by punching holes in the cover of a cardboard or tin box; a test tube holder can be improvised by attaching wires to a clothes peg; empty cleaned penicillin vials or other drug containers can be used for storing stains and chemicals; an empty tin can be used as a water bath; etc.

Appendix 1.2 is a list of reagents to be used in the laboratory.

Part II consists of the following chapters:

Chapter 7 : Examination of urine
Chapter 8 : Examination of stools
Chapter 9 : Examination of blood
Chapter 10 : Examination of sputum
Chapter 11 : Examination of skin smears and nasal smears
Chapter 12 : Examination of semen

Chapter 13 : Examination of throat swab
Chapter 14 : Examination of water sample

In this part of the Manual each laboratory investigation which you will be required to carry out is described—the equipment and reagents needed, the method of collecting the specimen, the steps of the procedure, the likely results and their interpretation.

The examinations which you will carry out provide important information to the doctors which will help them in deciding on the correct line of treatment for the patients. It is, therefore, most essential that every examination should be carried out with meticulous care, using the accurately measured quantities of reagents, employing the prescribed techniques and reporting the correct results.

The information received by you from the doctor about each patient, and the results of the tests carried out by you must be treated as *strictly confidential* and should only be given to the doctor requiring the examination.

For each investigation described in the Manual the normal findings and normal range of values are given.

Units of Measurement used in the Manual

Name of Quantity	*Unit*	*Symbol*
Length	metre	m
	centimetre	cm (1/100 m)
	millimetre	mm (1/1000 m)
	micron	μ (1/1,000,000 m)
Area	square metre	m^2
	square centimetre	cm^2
	square millimetre	mm^2
Volume (solid)	cubic metre	m^3
	cubic centimetre	cm^3
	cubic millimetre	mm^3
Volume (liquid)	litre	$l = 1000\ cm^3$
	centilitre	$cl = 10\ cm^3$
	millilitre	$ml = cm^3$
	microlitre	$\mu l = mm^3$
Weight	gram	g
	centigram	cg
	milligram	mg
Time	hour	h

Results of various laboratory investigations are expressed in different ways. For instance:

Investigation	*Result expressed as*	*Example*
Total red blood cell count	millions of RBC per cubic microlitre of blood	4.5 millions/mm^3
Total white blood cell count	number of WBC per cubic microlitre of blood	5000/mm^3
Haemoglobin	grams of Hb per 100 millilitres of blood	15.0 g/100 ml

Note: Haemoglobin can also be expressed as a percentage by converting the quantity into the equivalent percentage. For instance:

For males: 15.0 g/100 ml = 100% Hb
Hence 10.0 g/100 ml = 66.7% Hb

For females: 14.5 g/100 ml = 100% Hb
Hence 10 g/100 ml = 68.9% Hb

Job Responsibilities of Laboratory Technician

Note: All Primary Health Centres and Subsidiary Health Centres have been provided with a post of Laboratory Technician/Assistant. The Laboratory Technician will be under the direct supervision of the Medical Officer, Primary Health Centre.

The Laboratory Technician will carry out the following functions:

I. General Laboratory Procedures

1. Maintain the cleanliness and safety of the laboratory.
2. Ensure that the equipment and glassware are kept clean.
3. Handle and maintain the microscope.
4. Sterilize the equipment as required.
5. Dispose of specimens and infected material in a safe manner.
6. Maintain the necessary records of investigations done and submit the reports to the MO PHC.
7. Indent for supplies for the laboratory through the MO PHC and ensure the safe storage of materials received.
8. Prepare monthly reports regarding his work.

II. Laboratory Investigations

1. Carry out examination of urine
   - i. Specific gravity and pH
   - ii. Test for glucose
   - iii. Test for protein (albumen)
   - iv. Test for bile pigments and bile salts
   - v. Test for ketone bodies
   - vi. Microscopic examination
2. Carry out examination of stools
   - i. Macroscopic examination
   - ii. Microscopic examination
3. Carry out examination of blood
   - i. Collection of blood specimen by finger prick technique
   - ii. Haemoglobin estimation
   - iii. RBC count
   - iv. WBC count—total and differential
   - v. Preparation, staining and examination of thick and thin blood smears for malaria parasites and for microfilariae
   - vi. Erythrocyte Sedimentation Rate
4. Carry out examination of sputum
   - i. Preparation, staining and examination of sputum smears for Mycobacterium tuberculosis
5. Carry out examination of skin and nasal smears
   - i. Preparation, staining and examination of skin smears for Mycobacterium leprae
   - ii. Preparation, staining and examination of nasal smears for Mycobacterium leprae
6. Carry out examination of semen
   - i. Macroscopic examination
   - ii. Sperm count and motility
7. Prepare throat swabs
   - i. Collection of throat swab and examination for diphtheria bacilli
8. Test samples of drinking water
   - i. Collection of sample
   - ii. Testing of sample for gross impurities
   - iii. Testing of sample for free residual chlorine

PART I

GENERAL LABORATORY PROCEDURES

CHAPTER 1

Maintenance of Cleanliness of the Laboratory

You can carry out your work in a more efficient and safe manner if you always see that the laboratory is kept neat and clean, if you take certain precautions to ensure your own health and safety, and if you work with scrupulously clean equipment.

1. Maintenance of Cleanliness

(1) Keep the laboratory neat, clean and tidy.

(2) Arrange the laboratory so that you know exactly where to find each article. After use, replace chemicals and equipment in their proper places.

(3) While working, handle specimens, especially infectious material, carefully. Wash your hands thoroughly with soap and water and rinse them with disinfectant solution after carrying out the tests.

(4) During work use an apron to prevent contamination of the clothes and to avoid catching any infection.

(5) Avoid using a pipette by mouth. Use rubber bulbs on the pipette to suck up material. Haemocytometer pipettes which are used by mouth should be placed in a clean place.

(6) Smoking and eating food should not be permitted in the laboratory. If there is a refrigerator in the laboratory, it should not be used for storing any food.

(7) After finishing work, swab the working table with a disinfectant solution. Ensure that the floor is swept and swabbed with disinfectant solution every day.

(8) All contaminated liquid or solid material should be decontaminated before disposal. The contaminated material that is to be disposed of by burning at a site away from the laboratory should be placed in leak-proof durable containers which are closed before being taken away from the laboratory.

(9) If infected material drops on the bench, pour phenol on it and soak it up with newspaper which is then burnt.

2. Personal Health and Hygiene

As a Laboratory Technician, you will deal with examination and testing of various body fluids and materials which may be highly infectious. You will also come into close contact with people who have infectious diseases. Hence you must be very careful and take personal precautions during work. You should follow the steps given below:

(1) Physical fitness: Look after your health by taking proper diet and regular exercise, as you are prone to catch infection from the patients attending the laboratory.

(2) Immunization: You must take the necessary inoculations against Cholera, Typhoid fever and Tetanus.

(3) Hygiene: Keep your nails short and clean. Wash your hands frequently with soap and water.

3. Cleaning of Glassware

Always ensure that the glassware and the equipment in the laboratory are kept clean. This is necessary to obtain correct results of the tests carried out in the laboratory.

(1) Cleaning new glassware

Glassware that has never been used is slightly alkaline. In order to neutralize it:

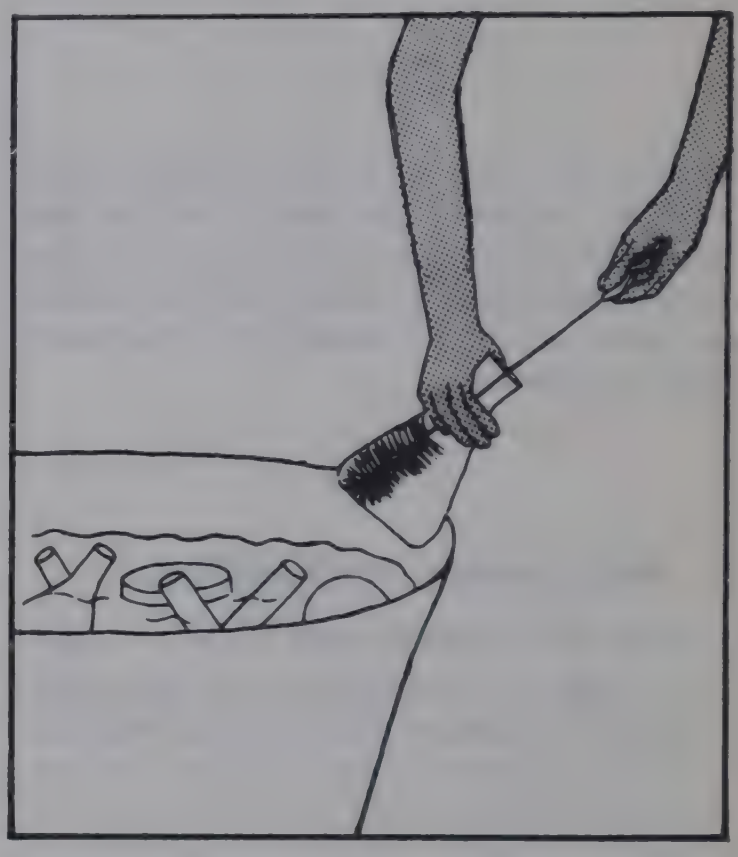
- prepare a basin containing 3 litres of water and 60 ml of concentrated hydrochloric acid (i.e. a 2% solution of acid)
- leave the new glassware completely immersed in this solution for 24 hours
- rinse twice with ordinary water and once with distilled water
- dry.

(2) Cleaning dirty glassware

i. Disposal of specimen containers: See Ch. 4.

ii. Preliminary rinsing: Rinse twice in cold or lukewarm water. Never rinse blood-stained tubes in hot water. Glassware that has been used for fluids containing protein must never be allowed to dry before first being rinsed and then washed.

iii. Soaking in detergent solution: Prepare a bowl of water mixed with washing powder or liquid detergent. Put the glassware in the bowl and brush the inside of the containers with a test tube brush. Leave to soak for 2 to 3 hours.



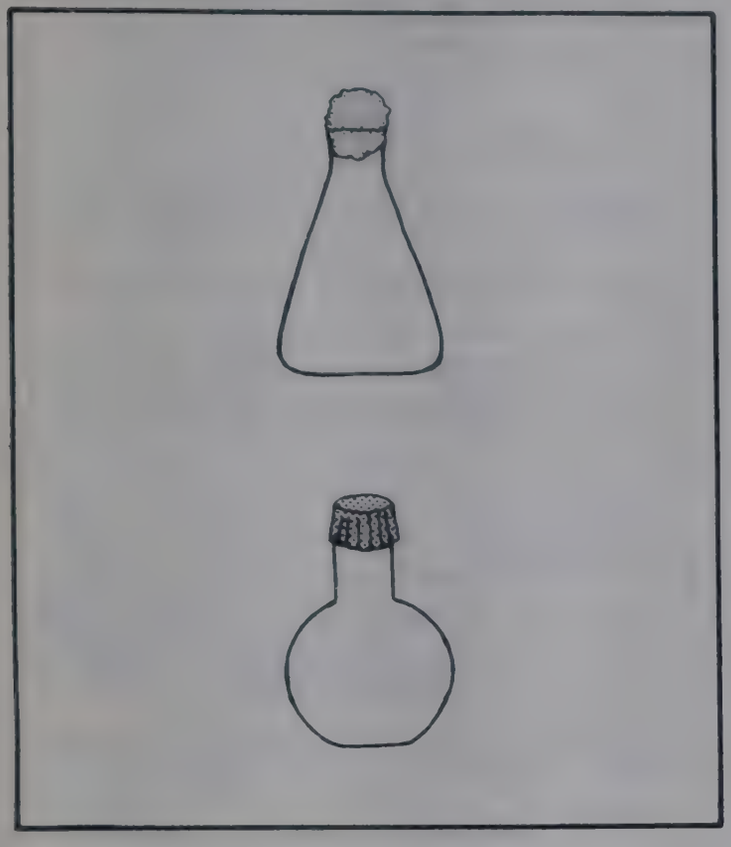
iv. Rinsing: Remove the articles one by one. Rinse each one thoroughly under the tap. Then soak them all in a bowl of ordinary water for 30 minutes. Rinse each article in a stream of clean water. *Remember*: that traces of detergent left on glassware can lead to false laboratory results.

v. Draining: Place glassware on the pegs of a wall draining rack or upside down in a wire basket.

vi. Drying: Place the basket in a sunny spot in the laboratory and cover it with a clean cloth.

vii. Plugging: The clean dry glassware should be put away in a cupboard to protect it from dust. The containers should be plugged with non-absorbent

cotton wool or the mouths covered with little caps made from clean paper.



(3) Cleaning pipettes

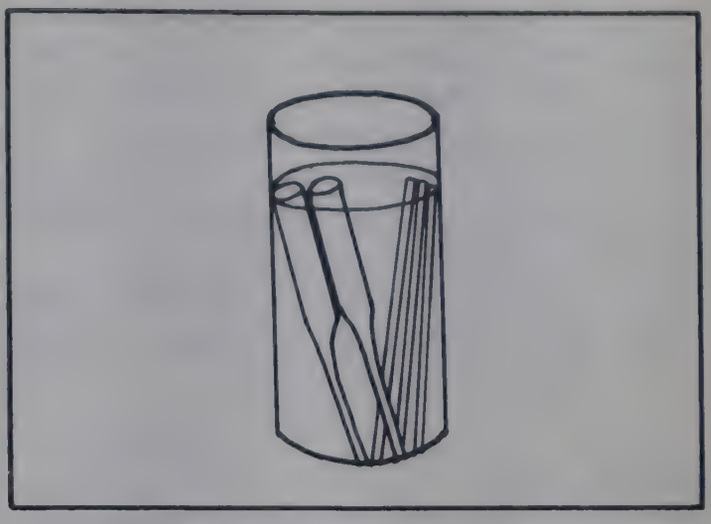
i. Immediate rinsing: As soon as a pipette has been used, it must be rinsed at once in a stream of cold water to remove blood, urine, reagents, etc.

ii. Soaking in water: After rinsing, place the pipettes in a large plastic cylinder or bowl full of water. If the pipettes have been used to measure infected material, leave them in a cylinder full of disinfectant solution (e.g. 2% phenol) for 24 hours.

iii. Soaking in detergent and rinsing: Follow steps iii & iv under item (2) above.

iv. Blocked pipettes:

(a) Put them in a cylinder filled with dichromate cleaning solution (Reagent No. 10). Slide them carefully into the solution and leave for 24 hours.



(b) The next day, pour the dichromate solution into another cylinder. It can be used 4 times.

(c) Hold the cylinder containing the pipettes under the tap and rinse thoroughly.

(d) Remove the pipettes one at a time. Check that the obstruction has been washed away. Rinse again.

(e) Leave to soak in ordinary water for 30 minutes, then repeat in clean water for 30 minutes.

Warning: The dichromate solution is highly corrosive and should be used with extreme care. If accidentally spilled on skin, eye or clothing, wash at once with copious amounts of water.

v. Drying: Dry pipettes in the air.

(4) Cleaning microscope slides

A. New slides

i. Soaking in detergent solution: Prepare a bowl of water with washing powder or liquid detergent using the amount of detergent recommended by the manufacturer. Place the slides in the bowl one by one and leave to soak overnight.

ii. Rinsing in water: Rinse each slide with tap water and then soak in clean water for 15 minutes.

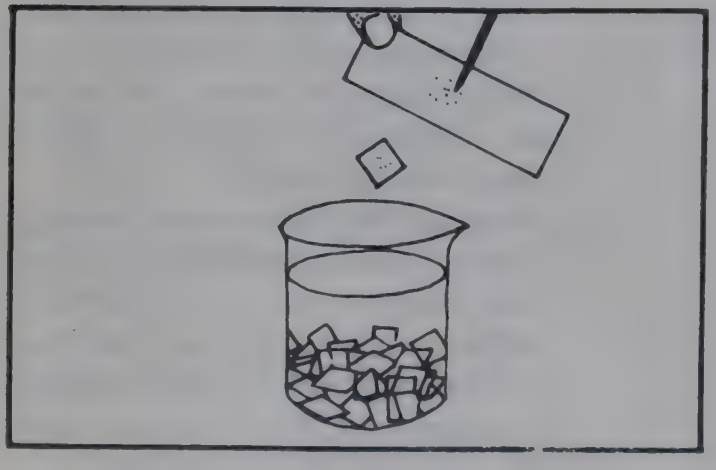
iii. Wiping and drying: Wipe the slides, one at a time, with a soft, non-fluffy cloth. Place them on a sheet of filter paper, one by one. Leave to dry. Examine each slide. Reject slides that are stained, scratched or yellow and those that have dull patches on them.

iv. Wrapping up: Divide the slides into piles of 10 or 20 and wrap in small sheets of paper.

B. Dirty slides

i. Slides soiled with immersion oil: Take the slides one by one and rub them with newspaper to remove as much of the oil as possible.

ii. Slides with coverslips: Using the tip of a needle or forceps, detach the coverslips and drop them into a beaker of water.



For cleaning of coverslips see Item (5) below.

iii. Soaking in concentrated detergent solution:
Prepare a bowl containing:
— cold or lukewarm water
— detergent in the amount recommended by the manufacturer
Leave to soak for 24 hours.
Detergents containing enzymes are excellent for removing blood films.
Note: When slides have been used for infected specimens (urine, stools), they should be placed in a disinfectant solution.

iv. Cleaning slides: Prepare another bowl containing a weak detergent solution (15 ml of domestic detergent per litre of water). Take the slides one by one out of the strong detergent solution. Rub each one with cotton wool dipped in the strong solution. Then drop it into the bowl of weak detergent. Leave to soak for 1 or 2 hours in the bowl of weak detergent.

v. Rinsing slides: Take the slides out one by one, preferably using forceps. If you use your fingers, pick them up by the edges. Rinse them separately under the tap. Then soak them for 30 minutes in a bowl of water. This is the best method.
Quick method: Empty the bowl of weak detergent solution and fill with clean water. Change the water 3 times, shaking the bowl vigorously each time.

vi. Wiping, drying and wrapping up: Follow the instructions given above for new slides.

(5) Cleaning coverslips

Coverslips can be recovered after use, cleaned and used again. For cleaning:

i. Soak in a weak detergent solution with added disinfectant made up in a large beaker as follows:
— 200 ml of water
— 3 ml of detergent
— 15 ml of bleaching powder
Leave to soak for 2 to 3 hours, shaking gently from time to time.

ii. Rinse out the beaker with tap water 4 times, shaking gently.

iii. Give a final rinse with distilled water.

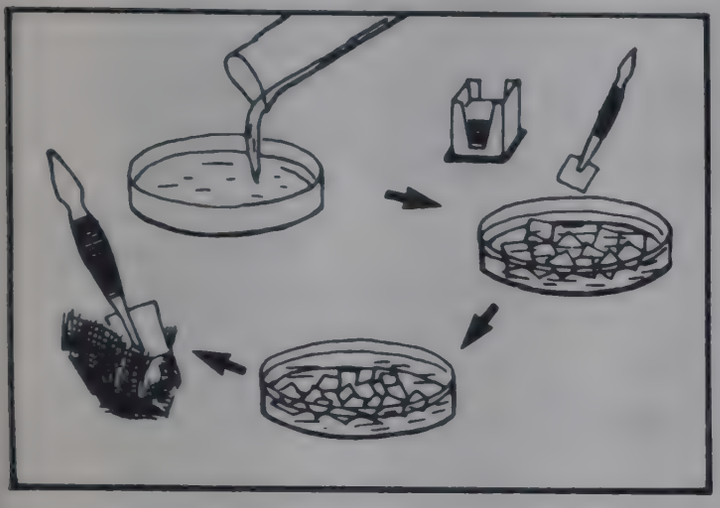
iv. Drain the coverslips by tipping them out carefully on to a pad of gauze.

v. Dry in air.

vi. Keep them in a small Petri dish. When taking them out, use special coverslip forceps, if possible.

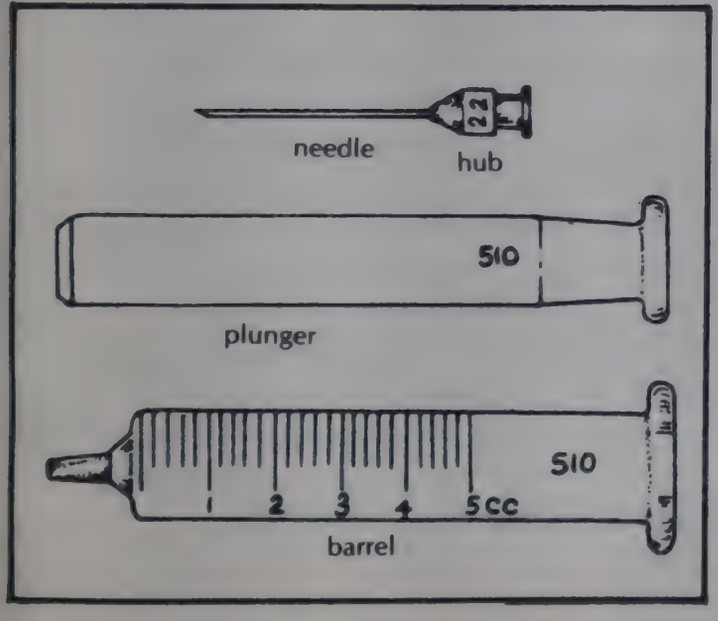
(6) Preparation of grease-free coverslips

i. Mix in a cylinder:
—10 ml 95% ethanol and 10 ml ether.
ii. Pour into a Petri dish and in it place 30 coverslips, one by one. Shake and leave for 10 minutes.
iii. Take the coverslips out one by one and dry them with gauze.
iv. Keep the coverslips in a dry Petri dish.

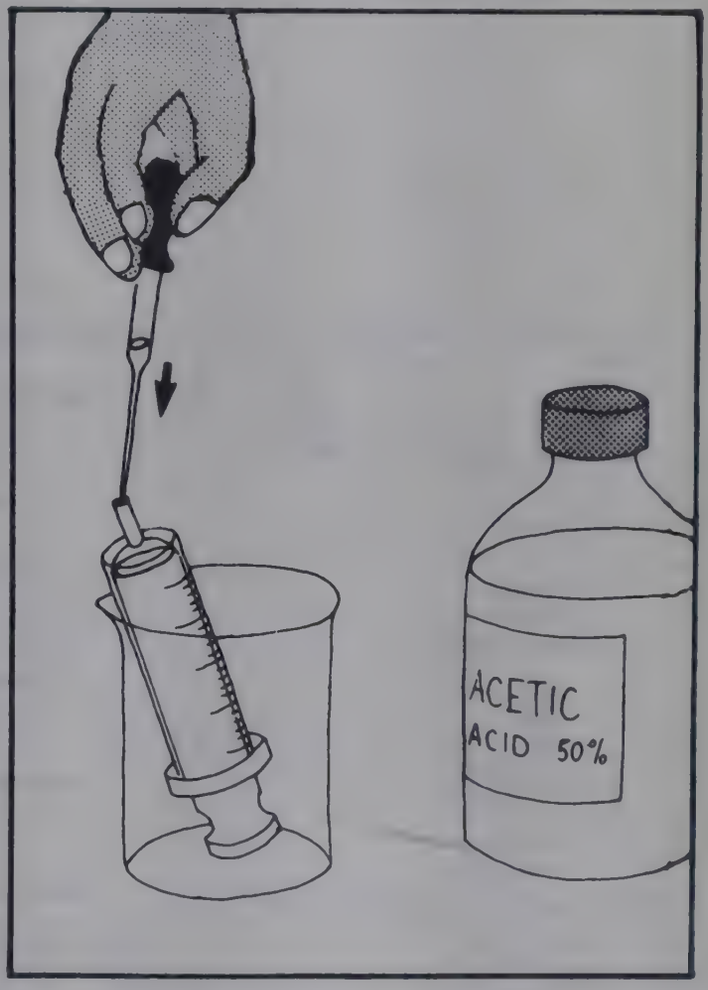


(7) Cleaning syringes and needles

As soon as a sample has been collected, remove the plunger and rinse the barrel and plunger. Fill the barrel and insert the plunger. Force the water through the needle. Finally, remove the needle and rinse the hub cavity.



i. Syringe with blocked piston: To loosen the piston, several methods may be used:
(a) Soak for 2 hours in hot water (about 70° C).
or (b) Pipette 50% acetic acid into the nozzle of the syringe with a fine Pasteur pipette. Stand the syringe on its end, piston down. Leave for 10 minutes.



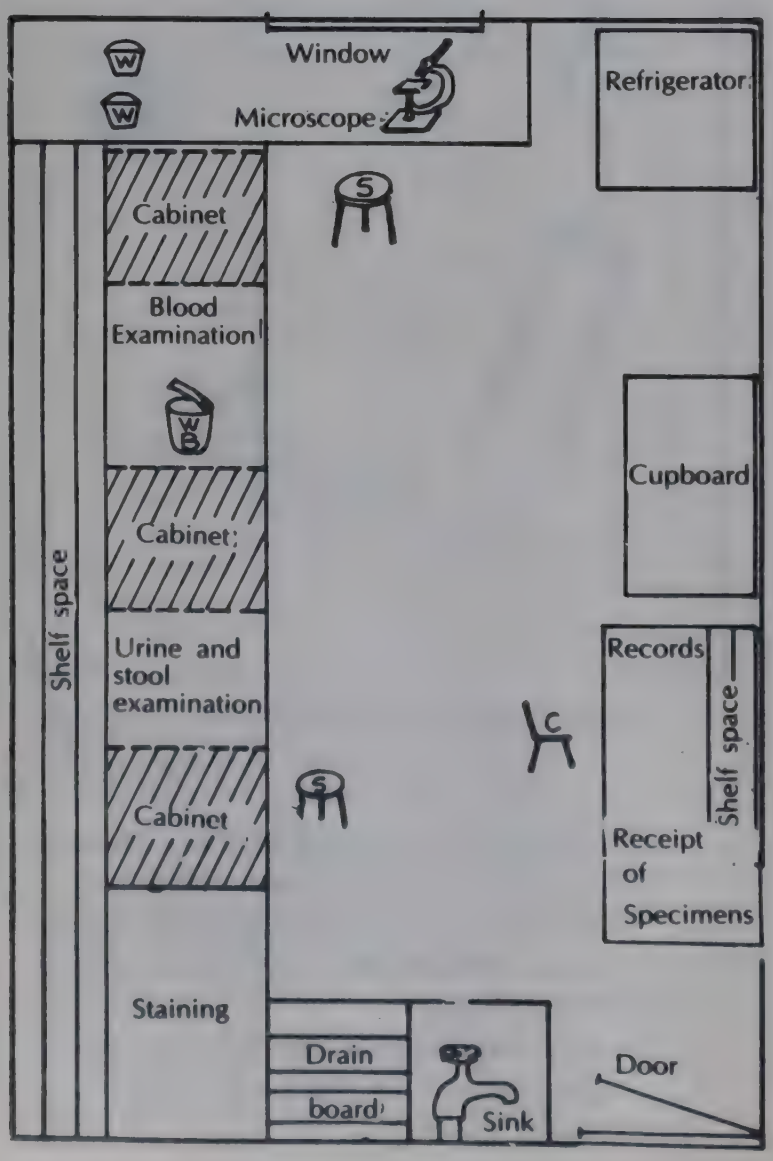
or (c) Soak the syringe for several hours in a bowl of 10 vol. hydrogen peroxide.
ii. Rinsing and soaking needles: As soon as the needle has been used, rinse it while it is still attached to the syringe and leave it to soak in the same way as the syringe.
iii. Blocked needles: Use a nylon thread dipped in 50% acetic acid. Otherwise use a stylet.

4. Arrangement of Laboratory

The plan below sets out the possible arrangement of a laboratory attached to a Primary Health Centre. It shows a laboratory capable of carrying out the techniques described in this Manual. The plan is limited to one room since this is all that is available for a laboratory at the PHC. The room should be at least 4.5 metres × 3 metres in size.

Appendix 1.1 is a suggested list of the apparatus needed to equip a laboratory capable of carrying out all the examinations described in this Manual.

Appendix 1.2 is a list of reagents to be used in the PHC laboratory. The composition of each reagent is given. Each reagent has a number which is mentioned whenever that reagent is referred to in any part of the Manual.



Scale: 2.5 cm = 1 metre

Suggested Plan for a Laboratory

Appendix 1.1: Suggested List of Equipment for PHC Laboratory

The following is a list of the apparatus needed to equip a laboratory capable of carrying out all the examinations listed in the Manual.

	Quantity
A. Furniture	
1. Laboratory table unit complete	1
– With formica top and knee space	
– Porcelain or stainless steel sink with drain board	1
– Storage cabinets fitted under laboratory table	3
– Reagent racks fitted above laboratory table	
2. Writing table	1
3. Steel almirah	1
4. Swivel chair adjustable without arms	1
5. Revolving stools (steel)	2
6. Waste bin	1
7. Warm cupboard with 40 watt light bulb for microscope (in humid climates)	1
B. Instruments/apparatus	
1. Microscope—with monocular inclined tube; revolving nosepiece; three objectives (x 10, x 40, x 100 oil immersion); eyepieces (x 5, x 10); mechanical stage; condenser; plano-concave mirror; wooden cabinet with key; plastic cover.	1
2. Hand-operated centrifuge with head for two 15 ml tubes, complete with fixing arrangements	1
3. Refrigerator	1
4. Small autoclave (heated by oil stove or butane gas)	1
5. Pressure cooker—large domestic type, 6 litres capacity	1
6. Urinometer (Sigma)	1
7. Sahli Haemoglobinometer with standards and glass tube	1
with: – Sahli pipettes 0.02 ml with rubber tubing and mouthpiece	5
8. Haemocytometer—Improved Neubauer ruled counting chamber	1
with:–Coverglasses optically plane for counting chamber	2
– Blood pipettes 0.05 ml with rubber tubing and mouthpiece	3
9. Erythrocyte Sedimentation Rate stand (Westergren)	1
with: – Westergren tubes (2.5 mm graduated from 0 – 200 mm)	10
10. Timer (0 – 60 min) with alarm	1
11. Chloroscope	1

C. Glassware

1.	Beakers, (Pyrex) – 50 ml	3
	– 100 ml	3
	– 250 ml	3
	– 500 ml	1
2.	Bottles – 2.5 ml & 5ml preferably plastic	20
	– white glass, 25 ml, with metal screw cap and rubber washer for specimens	20
	– wide-mouthed, all varieties, for urine collection	20
	– wide-mouthed, glass (2 litre) with glass stopper or bakelite screw cap	1
	– polythene, 2 litre	1
	– 5 ml, 10 ml, 20 ml empty vials of penicillin and other medicines	as many as possible
3.	Centrifuge tubes, conical, 15 ml	20
4.	Centrifuge tubes, conical, 15 ml graduated in 0.1 ml, with stoppers	4
5.	Conical testing glasses	3
6.	Coverslips, 20 × 20 mm (0.13 to 0.16 mm thick)	20 × 100
7.	Desiccator, 20 cm diameter	1
8.	Drop bottles, glass or plastic, 100 ml	10
9.	Drop bottles, amber glass, 100 ml	3
10.	Evaporating dish, 75 mm (75 ml)	2
11.	Flasks – Erlenmeyer: Pyrex, conical, wide-mouthed	
	– 100 ml	2
	– 250 ml	2
	– Florence: flat bottomed, round—250 ml	1
12.	Funnels, glass – 60 mm diameter	1
	– 90 mm diameter	1
13.	Glass cylinder, 60 cm high	1
14.	Glass rods, solid, 6 mm diameter	3
15.	Glass tubing, wall 1 to 1.5 mm, diameter 7–8 mm	1 kg
16.	Jars for staining (250 ml)	6
17.	Measuring cylinders, glass – 10 ml	2
	– 50 ml	2
	– 100 ml	2
	– 250 ml	2
	– 500 ml	1
	– 1000 ml	1
18.	Microscope slides, 75 × 25 mm (1.1–1.3 mm thick)	3 × 100
19.	Petri dishes, glass – 112 mm diameter	2
	– 156 mm diameter	2
20.	Pipettes, graduated from the top and not to the tip	
	– 1 ml (subdivided 0.01 ml)	2
	– 2 ml (subdivided 0.01 ml)	2
	– 5 ml (subdivided 0.1 ml)	2
	– 10 ml (subdivided 0.1 ml)	2

21. Pipettes, Pasteur (dropping)	
– non-calibrated	100
– calibrated	5
22. Reagent bottles, pyrex with glass stoppers	
– 250 ml	20
– 500 ml	10
– 1000 ml	2
23. Staining troughs – rectangular (20 slides)	3
– vertical (10 slides)	1
24. Staining tray, enamel (30 × 25 cm)	1
25. Test tubes, Pyrex, 150 × 16 mm	50
26. Wash bottles, plastic – 500 ml	2
– 1000 ml	2
27. Watch glasses, 50 mm diameter	2

D. Miscellaneous items (non-consumable)

1. Aluminium containers with screw caps (for packing specimen tubes or bottles)	6
2. Aluminium wire, 1 mm diameter	1 metre
3. Aprons	3
4. Asbestos gauze	2
5. Bowls, plastic, 50 × 30 cm	3
6. Bottle-cleaning and test tube-cleaning brushes (various sizes)	6
7. Bucket, plastic, 12 litres	1
8. Bunsen burner for use with butane gas	1
9. Butane gas cylinder	1
10. Cheatle's forceps	1
11. Containers (wooden/metal/plastic/fibreboard) for despatch of water samples	2
12. Corkscrew	1
13. Dusters	6
14. File	1
15. Forceps, stainless steel, for slides	1
16. Glass marking pencil	1
17. Incinerator	1
18. Kerosene stove	1
19. Lancets for taking capillary blood *or* Hagedorn needle in container	2
20. Loop holders	2
21. Metal tray (perforated)	1
22. Mortar and pestle	1
23. Rack for drying slides	1
24. Rubber bulbs – for cleaning microscope and pipettes	2
– for use with Pasteur pipettes (teats)	6
25. Saucepan or degchi with lid (30 cm)	1
26. Scalpel (Bard-Parker) with disposable blades (for leprosy)	1
27. Scissors (medium)	1

28. Scrubbing brush	1
29. Slide box, wooden, for 100 slides	1
30. Soap dish	1
31. Spatulas, various sizes, for reagent weighing	3
32. Spirit lamp (150 ml)	1
33. Stoppers, various sizes – cork	10
– rubber	10
34. Test tube holder	1
35. Test tube rack for 6 test tubes (15 × 1.8 cm)	2
36. Thermometer (0 – 100°C)	1
37. Tripod	1
38. Towels	6
39. Wall rack for draining glassware	1
40. Wire basket	1
41. Wire for loops, 0.46 mm (nickel-chromium alloy)	1 metre
42. Wooden block for loopholders	1

E. Stationery and laboratory records

1. Examination Request Forms	1000
2. Registers	2
3. Report Forms	200 of each
4. Monthly Report Forms	30
5. Adhesive tape	1 roll
6. Blotting paper	12 sheets
7. Brown paper	12 sheets
8. Cellotape	1 roll
9. Corrugated paper	1 roll
10. Gum	1 bottle
11. Labels for bottles	200
12. Office files	2
13. Paintbrush	1
14. Paper, ruled, quarto size	1 ream
15. Pens, ballpoint – black or blue ink	4
– red ink (for recording positives)	2
16. Pencils – glass marking wax – blue	3
– glass marking wax – red	3
– lead	12
– red and blue	2
– skin	1
17. Rubber bands	25
18. Ruler (30 cm/12")	1
19. Stapler	1
20. Staples	1 box (1000)
21. String	1 ball

F. Other consumable items

1. Applicators, wooden, 12 cm × 1 mm (can be made locally)	50
2. Cartons (plastic or waxed paper cups), disposable for stool & sputum collection	100
3. Cotton swab sticks	50
4. Cotton wool – white absorbent	1 roll × 500 g
– white non-absorbent	1 roll × 500 g
5. Detergent – powder	2 kg
– liquid	2 litres
6. Disinfectant – Lysol/Phenol 5%	2 litres
– Commercial bleaching powder 10%	2 kg
7. Filter paper 15 cm No. 1	2 boxes
8. Litmus paper – blue	4 books
– red	4 books
9. Matches	12 boxes
10. Nylon thread (000)	1 metre
11. pH paper – narrow range: 5–7	4 books
– narrow range: 6–8	4 books
– wide range: 1–10	4 books
12. Plastic packets (16 × 14 cm)	12
13. Quicklime (washing soda)	2 kg
14. Soap	6 cakes
15. Soft non-fluffy cloth and clean rags	as many as possible
16. Stylets	12
17. Tongue depressors, wooden	50
18. URISTIX	2 bottles × 50
19. Washing powder	2 kg

G. Chemicals

1. Acetic acid, glacial
2. Acetone
3. Ammonia
4. Basic fuchsin
5. Distilled water
6. Ethanol 95% (ethyl alcohol)
7. Ether
8. Hydrochloric acid (commercial)
9. Hydrogen peroxide, 10 vol
10. Immersion oil
11. Methylated spirit
12. Methanol (methyl alcohol)
13. Petroleum jelly (vaseline)
14. Silica gel (blue)
15. Sodium bicarbonate
16. Sodium dichromate solution
17. Sodium nitroprusside crystals
18. Sulphur flowers powder
19. Sulphuric acid (conc)
20. Tincture benzoin
21. Xylene

Appendix 1.2: Reagents and their Preparation

Reagents are listed in alphabetical order. For exarnple:

acetic acid	. . is under . . A
Benedict qualitative solution	. . is under . . B
carbol fuchsin	. . is under . . C
hydrochloric acid	. . is under . . H
sodium chloride	. . is under . . S

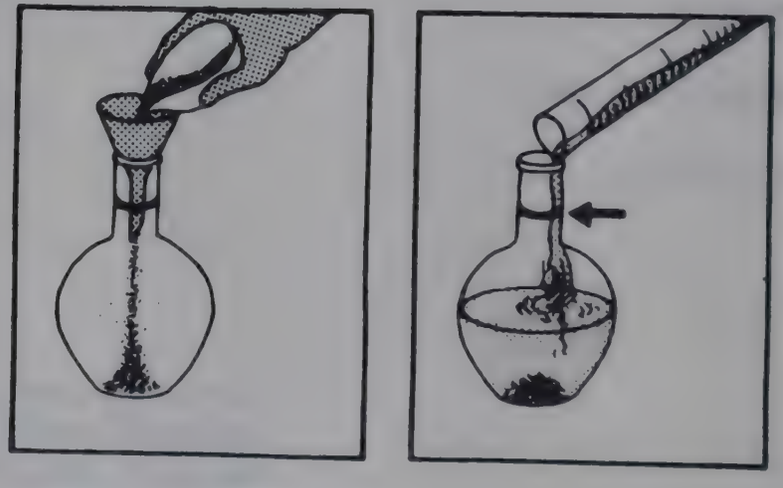
Each reagent has a number. These numbers are referred to in the techniques.

q.s = the quantity required to make up a certain volume

For example: sodium chloride . . 8.5 g
distilled water . . q.s. 1000 ml

This means:

Place 8.5 g sodium chloride in a volumetric flask or measuring cylinder. Add enough water (quantity sufficient or q.s.) to obtain a total volume of 1000 ml.



In most cases the chemical formulae of the compounds used are given immediately after the English names:

— sodium chloride (NaCl)
— sulphuric acid (H_2SO_4)
etc.

This can be useful when checking the label on the bottle.

An *aqueous solution* is a solution of a compound dissolved in water.

1. ACETIC ACID, 100 g/l (10%)

Glacial acetic acid (CH_3COOH)	20 ml
Distilled water	q.s. 200 ml

Warning: Glacial acetic acid is highly corrosive.

2. ACID-ETHANOL

For modified Ziehl-Neelsen stain

Hydrochloric acid	3 ml
70% Ethanol	upto 100 ml

Warning: Hydrochloric acid is highly corrosive.

3. BARIUM CHLORIDE, 100 g/l (10%) AQUEOUS SOLUTION

Barium chloride ($BaCl_2$)	10 g
Distilled water	q.s. 100 ml

4. BENEDICT QUALITATIVE SOLUTION

Copper sulphate ($CuSO_4.5H_2O$)	17.3 g
Trisodium citrate ($Na_3C_6H_5O_7.2H_2O$)	173.0 g
Sodium carbonate (Na_2CO_3), anhydrous	100.0 g
Distilled water	1000 ml

Dissolve the copper sulphate crystals by heat in 100 ml distilled water. Dissolve the trisodium citrate and the sodium carbonate in about 800 ml water. Add the copper sulphate solution slowly to the sodium carbonate/trisodium citrate solution, stirring constantly. Make up the mixture to 1000 ml with distilled water.

5. BUFFERED WATER for Giemsa and Leishman stains

Disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$)	3.76 g
Potassium dihydrogen phosphate (KH_2PO_4), anhydrous	2.1 g
Distilled water	q.s. 1000 ml

Check the pH using narrow-range pH papers. The pH should be 7.0–7.2.

6. BUFFERED WATER for JSB stain

Disodium hydrogen phosphate	0.417 g
Potassium acid phosphate	0.752 g
Distilled water	2000 ml

(pH 6.2–6.8)

7. CARBOL FUCHSIN

A. For Ziehl-Neelsen stain

Solution (a):

Saturated solution of basic fuchsin

Basic fuchsin	3 g
Ethanol, 95%	100 ml

Solution (b):

Phenol solution, 50 g/l (5%), aqueous	
Phenol	10 g
Distilled water	q.s. 200 ml

Then:

Solution (a)	10 ml
Solution (b)	90 ml

Warning: This solution is highly corrosive and poisonous.

B. For modified Ziehl-Neelsen stain

Basic fuchsin	1 g
95% Ethanol	10 ml
5% Phenol solution	90 ml

8. CARY-BLAIR TRANSPORT (HOLDING) MEDIUM

Sodium thioglycolate	1.5 g
Disodium hydrogen phosphate (Na_2HPO_4), anhydrous	1.1 g
Sodium chloride	5.0 g
Agar	5.0 g
Distilled water	991.0 ml

i. If possible, prepare in chemically clean glassware.
ii. Heat while mixing until the solution just becomes clear.
iii. Cool to 50°C, add 9 ml of freshly prepared aqueous calcium chloride solution, 10 g/l (1%), and adjust the pH to about 8.4.
iv. Pour 7 ml into previously rinsed and sterilized 9 ml screw-capped vials.
v. Steam the vials containing the media for 15 minutes, cool, and tighten the caps.

9. CRYSTAL VIOLET, MODIFIED HUCKER

Solution A:

Crystal violet (certified)	2 g
Ethanol, 95%	20 ml

Solution B:

Ammonium oxalate [$(NH_4)_2\ C_2O_4\ H_2O$]	0.8 g
Distilled water	80.0 ml

Mix solutions A and B. Store for 24 hours before use. Filter through paper into staining bottle.

10. DICHROMATE CLEANING SOLUTION

For cleaning glassware.

Potassium dichromate ($K_2Cr_2O_7$)	100 g
Water	1000 ml
Pure sulphuric acid (H_2SO_4)	100 ml

Dissolve the dichromate in the water. Add the acid little by little, very carefully, stirring constantly. The acid must always be added to the water, NOT the water to the acid. If commercial detergents such as Teepol are available, dichromate cleaning solution is not usually necessary.

Warning: Since potassium dichromate and sulphuric acid are both corrosive and the mixture even more so, avoid using the solution as much as possible.

1. EDTA DIPOTASSIUM SALT SOLUTION, 100 g/l (10%) ('potassium edetate')

Dipotassium ethylenediaminetetraacetate		20 g
Distilled water	q.s.	200 ml

For use, pipette 0.04 ml of this solution into small containers marked to hold 2.5 ml of blood. Allow the anticoagulant to dry by leaving the containers overnight on a warm bench or in a 37° C incubator.

2. EOSIN, 20 g/l (2%) SOLUTION IN SALINE

Eosin		2 g
Sodium chloride, 8.5 g/l (0.85%) aqueous solution	q.s.	100 ml

3. FOUCHET REAGENT

(1) First prepare a 10% solution of iron ('ferric') chloride:

Iron chloride ($FeCl_3$)		10 g
Distilled water	q.s.	100 ml

(2) Preparation of reagent:

Iron chloride solution	10 ml
Trichloroacetic acid (CCl_3COOH)	25 g
Distilled water	100 ml

Dissolve the acid in about 70 ml of distilled water in a 100 ml volumetric flask. Add 10 ml of the 10% iron chloride solution. Make the volume up to 100 ml with distilled water.

Warning: Trichloroacetic acid is highly corrosive.

14. GIEMSA STAIN

Powdered Giemsa stain	0.75 g
Methanol (CH_3OH)	65 ml
Glycerol	35 ml

Put the ingredients in a bottle containing glass beads and shake. Shake the bottle 3 times a day for 4 consecutive days. Filter.

Check the manufacturer's instructions in case the quantities indicated are not the same.

15. GRAM IODINE SOLUTION

Iodine	1 g
Potassium iodide (KI)	2 g
Distilled water	300 ml

Grind the dry iodine and potassium iodide in a mortar. Add water, a few ml at a time, and grind thoroughly after each addition until the iodine and iodide dissolve. Rinse the solution into an amber glass bottle with the remainder of the distilled water.

Alternatively:

Measure 100 ml of water in a cylinder. First dissolve the potassium iodide in about 30 ml of the water. Add the iodine and mix until dissolved. Add the remainder of the water and mix well. Store in a brown bottle.

16. HYDROCHLORIC ACID, 0.1 mol/l (0.1 N)

Hydrochloric acid (HCl), concentrated	8.6 ml
Distilled water	q.s. 1000 ml

Measure out 500 ml of water. Add the acid drop by drop. Make up to 1 litre with the rest of the water. The solution obtained may be used only for haemoglobin estimation by the Sahli method. Renew monthly.

Warning: Hydrochloric acid is highly corrosive.

17. JASWANT SINGH & BHATTACHARYA (JSB) STAIN

JSB Solution I:

Methylene blue (medicinal)	0.5 g
Sulphuric acid, 1%	3.0 ml
Potassium dichromate	0.5 g
Disodium hydrogen phosphate dihydrate	3.5 g
Distilled water	500 ml

Dissolve methylene blue in distilled water. Add sulphuric acid gradually, 1 ml at a time, with constant stirring to ensure thorough mixing.
Add potassium dichromate. This forms a purple precipitate.
Add disodium hydrogen phosphate dihydrate. After stirring for some time the precipitate appears to get dissolved.
Boil the solution in a flask with a reflux condenser *for one hour when the blue colour of the solution deepens.* (Count the time from the exact time when the solution starts boiling.)

JSB Solution II:

Eosin (yellow, zinc free)	1.0 g
Distilled water	500 ml

18. LEISHMAN STAIN

Leishman powder	1.5 g
Methanol	q.s. 1000 ml

Rinse out a clean bottle with methanol. Add a few clean dry glass beads. Add the staining powder and methanol. Mix well to dissolve the stain. The stain is ready for use the following day.
When preparing an ethanol Romanowsky stain such as Leishman, it is important to allow no moisture to enter the stain during its preparation or storage.

19. LUGOL IODINE SOLUTION

Iodine	1 g
Potassium iodide (KI)	2 g
Distilled water	q.s. 100 ml

Weigh the iodine in a porcelain dish or a watch glass. Grind the dry iodine and potassium iodide in a mortar. Add water, a few millilitres at a time, and grind thoroughly after each addition until the iodine and iodide dissolve. Put the solution into an amber glass bottle with the remainder of the distilled water.

Alternatively:
Measure 100 ml of water in a cylinder. First dissolve the potassium iodide in about 30 ml of the water. Add the iodine and mix until dissolved. Add the remainder of the water and mix well. Store in a brown bottle.

20. LUGOL IODINE SOLUTION, 50 g/l (5%)

Iodine	5 g
Potassium iodide (KI)	10 g
Distilled water	q.s. 100 ml

Prepare in the same way as Lugol iodine solution (No.19). Store in a brown bottle for no more than one month.

21. METHYLENE BLUE, AQUEOUS

A. For Ziehl-Neelsen stain

Methylene blue	0.3 g
Distilled water	100 ml

Filter after dissolving.

B. For modified Ziehl-Neelsen stain

Methylene blue	0.5 g
Borax	5.0 g
Distilled water	100 ml

22. ORTHOTOLIDINE REAGENT

Orthotolidine dihydrochloride	1.35 g
Concentrated hydrochloric acid	150 ml
Distilled water	850 ml

Dissolve orthotolidine dihydrochloride in 500 ml distilled water (Solution 1). Make a mixture of 350 ml distilled water and 150 ml concentrated hydrochloric acid (Solution 2).
Add Solution 1 to Solution 2 with constant stirring.
Store the reagent in amber-coloured bottles.

23. RBC DILUTING FLUID (FORMALDEHYDE CITRATE)

Sodium citrate	3.0 g
Commercial formaldehyde solution, at least 37% ('formalin')	1.0 ml
Distilled water	100 ml

Warning: Formaldehyde is corrosive and poisonous.

24. SAFRANINE SOLUTION

Stock solution:

Safranine 0 (certified)	2.5 g
Ethanol, 95%	q.s. 100 ml

Working solution:

Stock solution	10 ml
Distilled water	90 ml

25. SODIUM CHLORIDE SOLUTION, 8.5 g/l (0.85%) ('isotonic saline')

Sodium chloride	8.5 g
Distilled water	q.s. 1000 ml

26. SODIUM THIOSULPHATE, 30 g/l (3%) AQUEOUS SOLUTION

Sodium thiosulphate, anhydrous (or an equivalent quantity of $Na_2S_2O_3.5H_2O$)	3 g
Distilled water	q.s. 100 ml

Store in a brown drop bottle.
Used to neutralize any chlorine in water samples taken for bacteriological analysis.

27. SPERM DILUTING FLUID (FORMALIN-BICARBONATE)

Sodium bicarbonate	5.0 g
Commercial formaldehyde solution (formalin)	1.0 ml
Distilled water	100 ml

28. WBC DILUTING FLUID (TÜRK SOLUTION)

Acetic acid (CH_3COOH), glacial	4 ml
Distilled water	q.s. 200 ml
Aqueous methylene blue solution	10 drops

The methylene blue solution is prepared by dissolving 0.3 g methylene blue in 100 ml of distilled water. Filter before adding to the acid solution.

Warning: Acetic acid is corrosive.

29. WILLIS SOLUTION

This is a saturated solution of sodium chloride.

Sodium chloride	125 g
Distilled water	500 ml

Dissolve the sodium chloride by heating the mixture to boiling point. Leave to cool and stand. Check that some of the salt remains undissolved. If it has all dissolved add a further 50 g. Filter and keep in a corked bottle.

CHAPTER 2

The Microscope

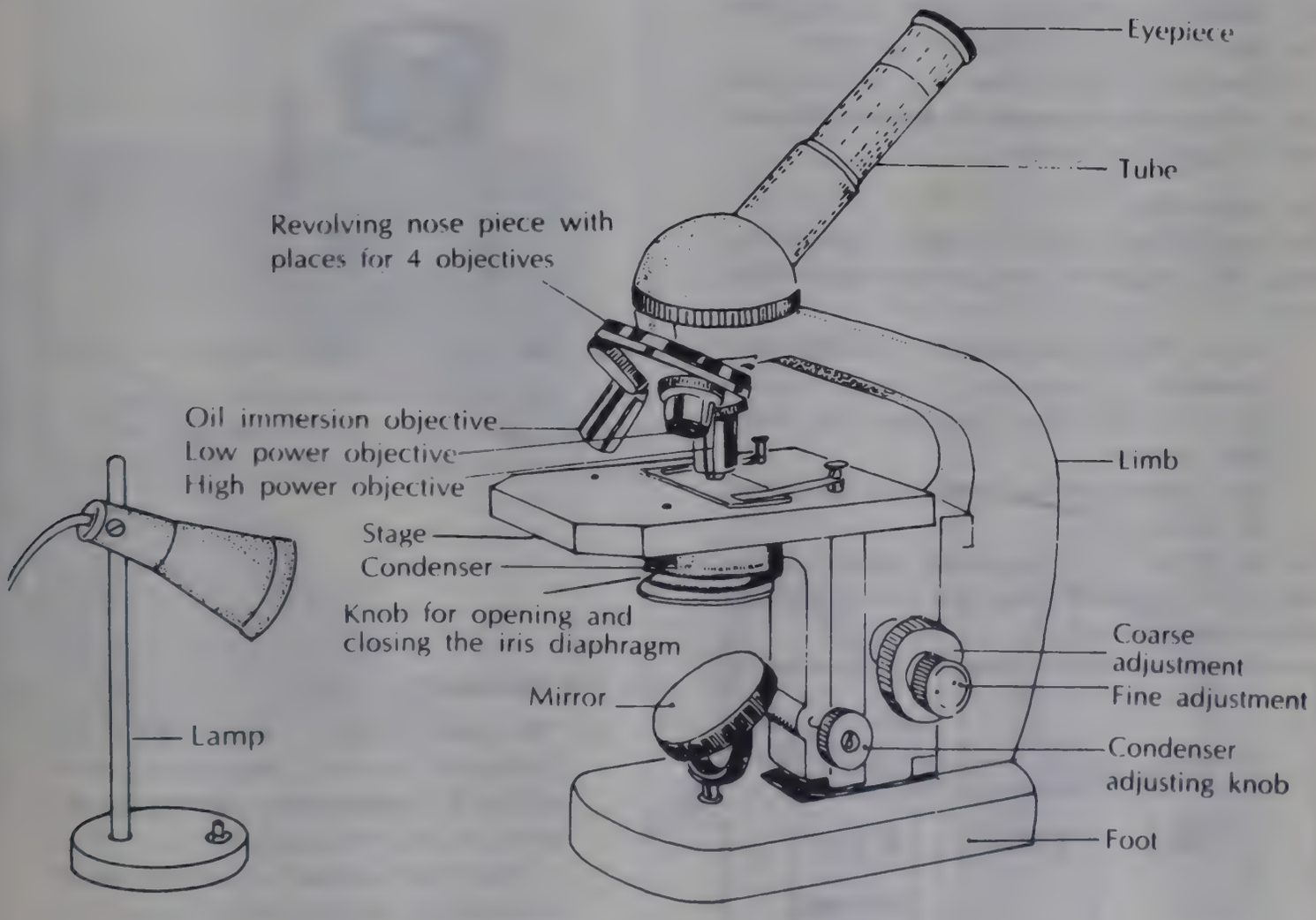
Many of the diseases prevalent in the rural areas are communicable diseases, transmitted by organisms that can often be seen under the microscope in specimens taken from patients. Direct microscopy is therefore indispensable in the PHC laboratory.

You will be provided with a microscope at the PHC. In order that the microscope may be effectively used, it is very important for you to know how to handle the microscope and how to maintain it properly.

1. How to Handle the Microscope

You must be familiar with the different parts of the microscope. These are as follows:

A. The support system (body)
B. The magnification system (lenses)
C. The illumination system (lighting)
D. The adjustment system (focusing light and movement)



A. The support system

This consists of:

(1) The foot
(2) The limb
(3) The revolving nosepiece (objective changer)
(4) The stage
(5) The mechanical stage, which gives a slow controlled movement to the object slide.

B. The magnification system

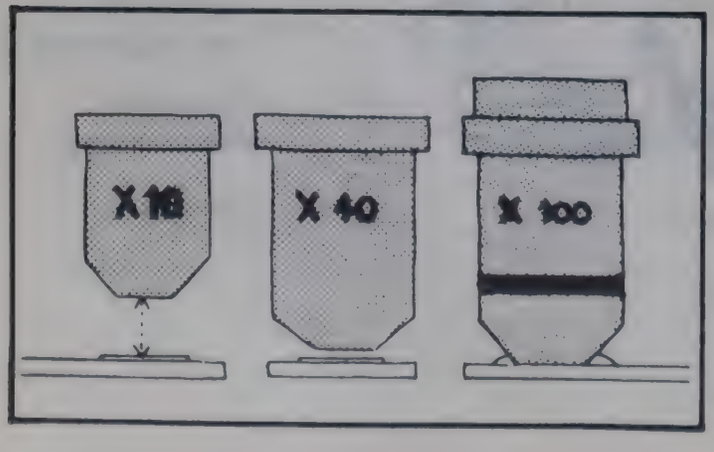
This consists of a system of lenses. The lenses of the microscope are mounted in two groups, one at each end of a long tube—the body tube. The first group of lenses is at the bottom of the tube, just above the preparation under examination (the object), and is called the *objective*.

The second group of lenses is at the top of the tube where the microscopist applies his eye, and is called the *eyepiece*.

The objectives:
The magnifying power of each objective is shown by a figure engraved on the sleeve of the lens:

– the × 10 objective magnifies 10 times (low power)
– the × 40 objective magnifies 40 times (high power)
– the × 100 objective magnifies 100 times (oil immersion)

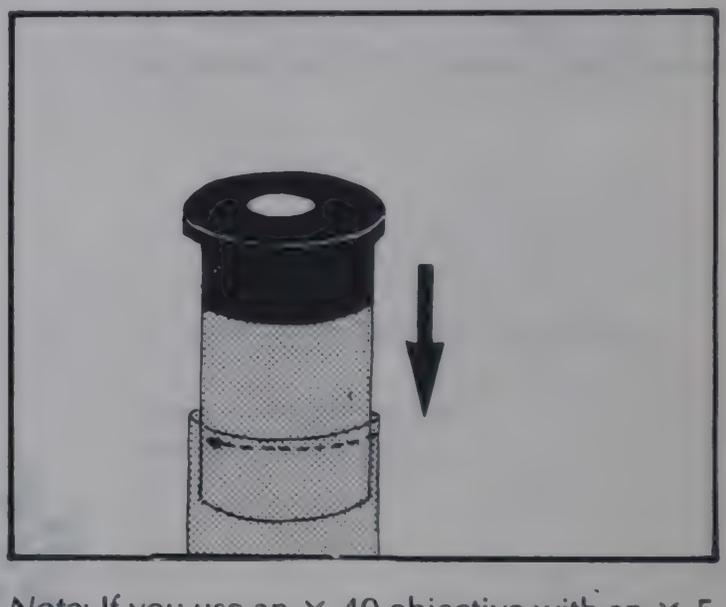
The × 100 objective is usually marked with a red or black ring to show that it must be used with immersion oil.



The greater the magnifying power of the objective, the smaller the working distance, i.e. the distance between the front lens of the objective and the object slide when the image is in focus.

The eyepiece:
The magnifying power of the eyepiece is marked on it:

– An × 5 eyepiece magnifies the image 5 times
– An × 10 eyepiece magnifies the image 10 times



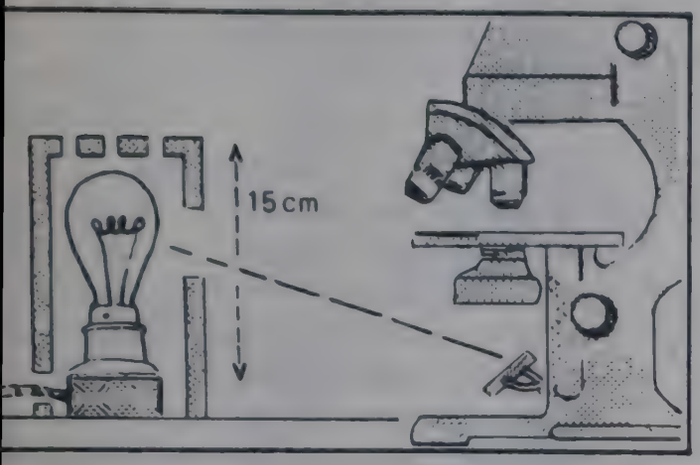
Note: If you use an × 40 objective with an × 5 eyepiece, the object will be magnified 40 × 5 times, i.e. the total magnification will be 200.

C. The illumination system

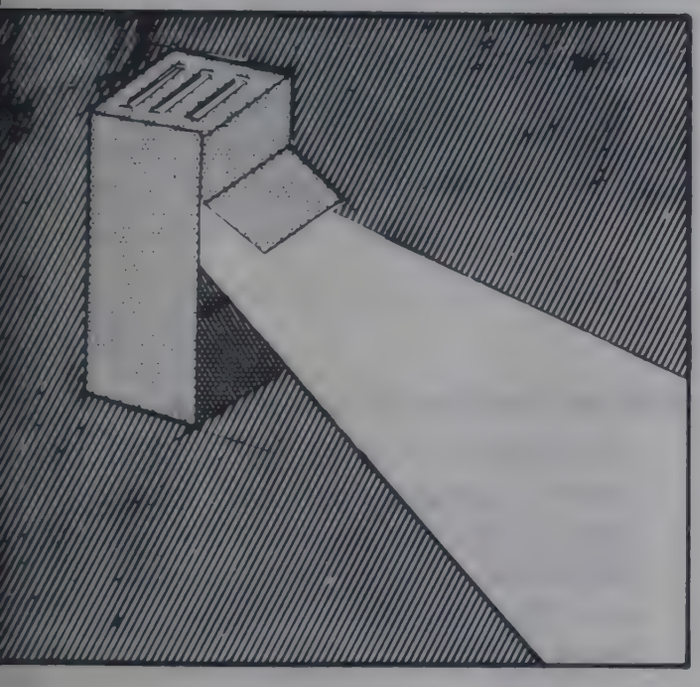
(1) The source of light: Electric light should preferably be used, since it is easier to adjust. It is provided by a lamp placed in front of the microscope.

You can make your own lamp for the microscope. A porcelain holder for a light bulb is fixed on a wooden base and the whole is encased in a wooden or tin box with an opening for the light.

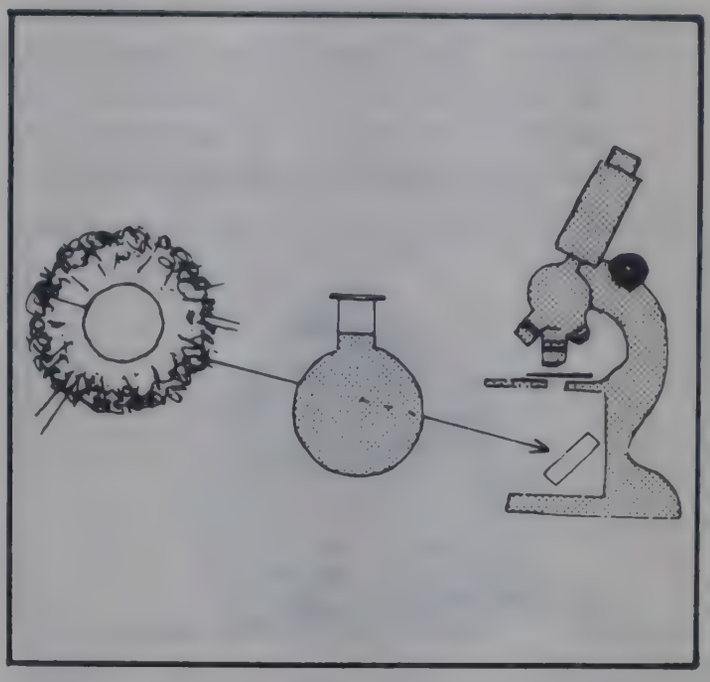
Cut slits in the top of the box to enable the bulb to cool;



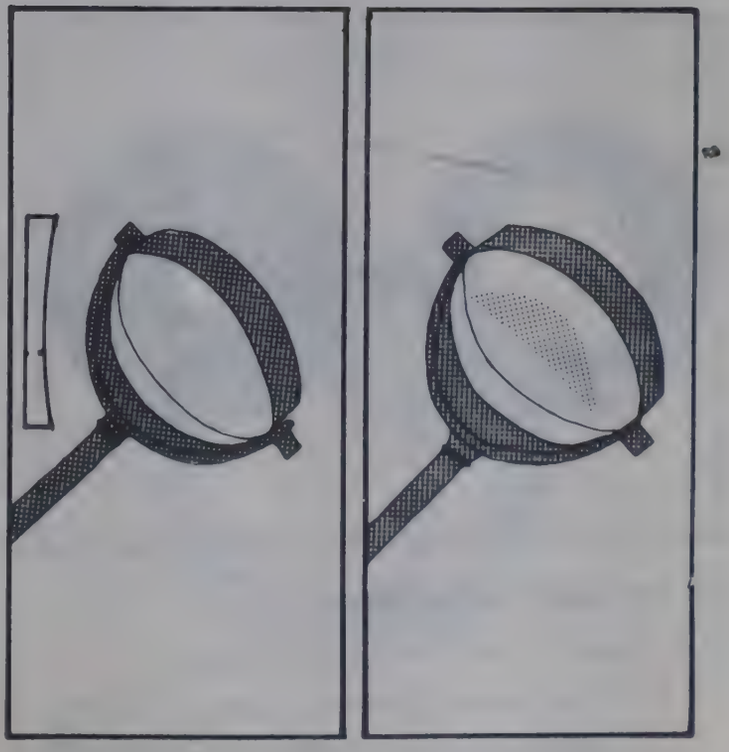
or
a flap can be fitted above the opening to serve as a shutter.



Use an opaque electric bulb of the 'daylight' type (blue-white) — 60 watts. Otherwise, daylight can be used. The microscope should never be placed in direct sunlight. It should be well illuminated but used in a subdued light. If there is bright sunlight a bottle or a round flask of clear glass and full of water can be placed in front of the microscope to reduce the intensity of the light.

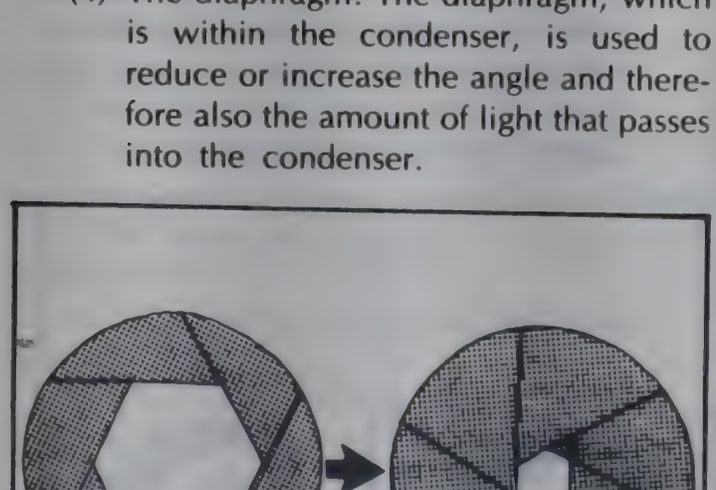


(2) The mirror: The mirror reflects rays from the light source on to the object. One side has a plane surface, the other a concave surface. The concave side forms a low-power condenser and is used if there is no condenser.



(3) The condenser: The condenser brings the rays of light to a common focus on the object to be examined. It is situated between the mirror and the stage. It can be raised (maximum illumination) and lowered (minimum illumination). It must be centred and adjusted correctly.

(4) The diaphragm: The diaphragm, which is within the condenser, is used to reduce or increase the angle and therefore also the amount of light that passes into the condenser.



D. The adjustment system

The system comprises the following:

(1) The coarse adjustment screw: This is the largest screw. It is used first to achieve an approximate focus.

(2) The fine adjustment screw: This move the objective more slowly. It is used t bring the object into perfect focus.

(3) The condenser adjustment screw: Thi is used to raise the condenser for greate illumination or to lower it to reduce th illumination.

(4) Condenser centring screws: There ma be 3 screws placed around th condenser—one in front, one on the lef and one on the right. They are used t centre the condenser exactly in relatio to the objective.

(5) The iris diaphragm lever: This is a smal lever fixed on the condenser. It can b moved to close or open the diaphragm thus reducing or increasing both the angle and the intensity of the light.

(6) Mechanical stage controls: These are used to move the object slide on the stage—one screw to move it backwards and forwards; and one screw to move i to left or right.

Points to remember in using the microscope

(1) Place the microscope on a firm, level bench of adequate size but not too high. I electric illumination is to be used, the microscope must be placed in the shade away from the window. Place a piece o heavy cloth under the microscope.

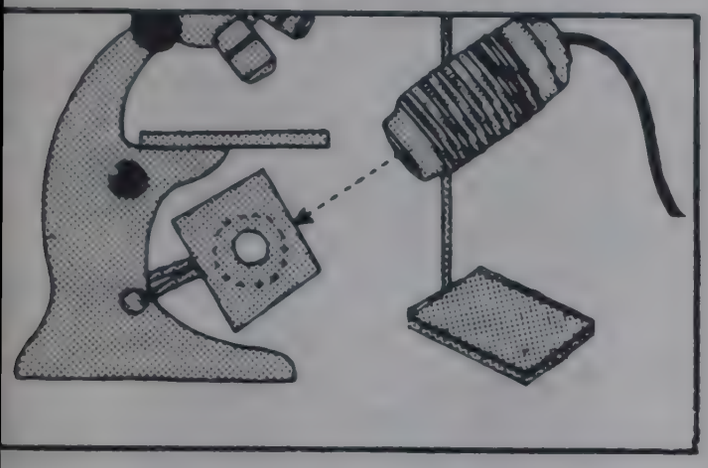
(2) Screw the objectives into the revolving nosepiece following this order in a clock wise direction:
- × 10 objective
- × 40 objective
- × 100 objective

(3) Place the eyepiece in the body tube.

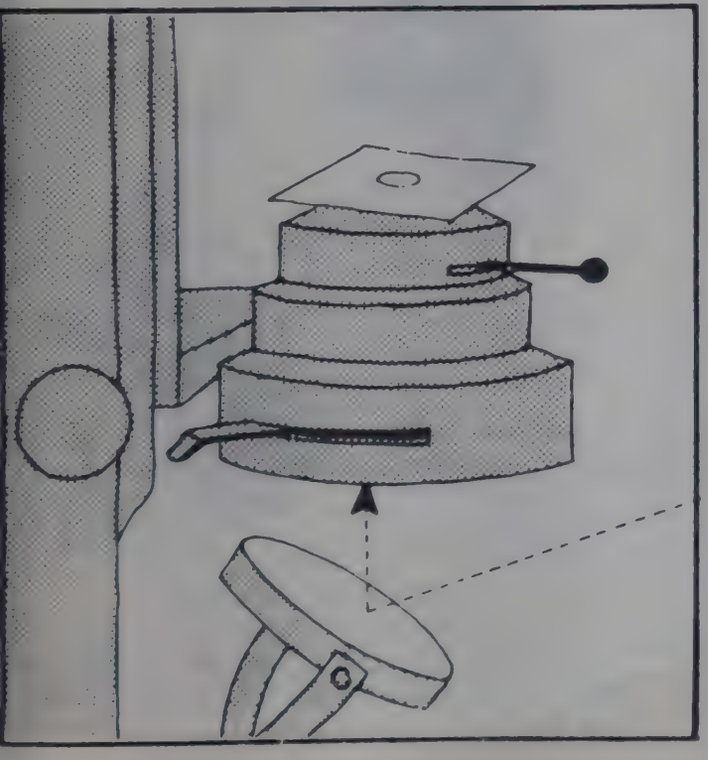
(4) Fix the condenser under the stage.

(5) Fix the mirror on the foot.

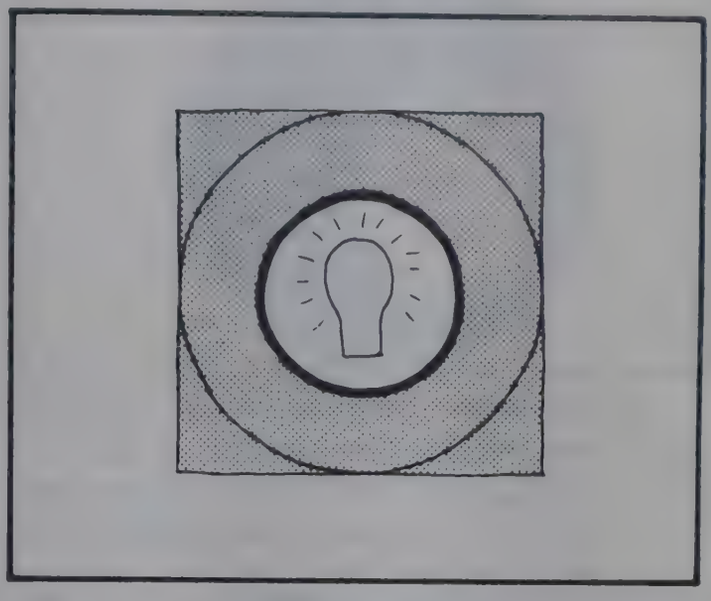
(6) If electric illumination is to be used, place the lamp 20 cm in front of the microscope facing the mirror, which should be fixed at an angle of 45°. Place a piece of paper over the mirror. Adjust the position of the lamp so that it shines on the centre of the mirror.



(7) Use the plane side of the mirror. Open the iris diaphragm to the maximum. Raise the condenser. Place a piece of thin white paper over the lens at the top of the condenser. This piece of paper should show an image of the electric bulb, surrounded by a circle of light.



Adjust the mirror so that the image of the bulb is in the exact centre of the circle of light (or the brightest part if daylight is being used).

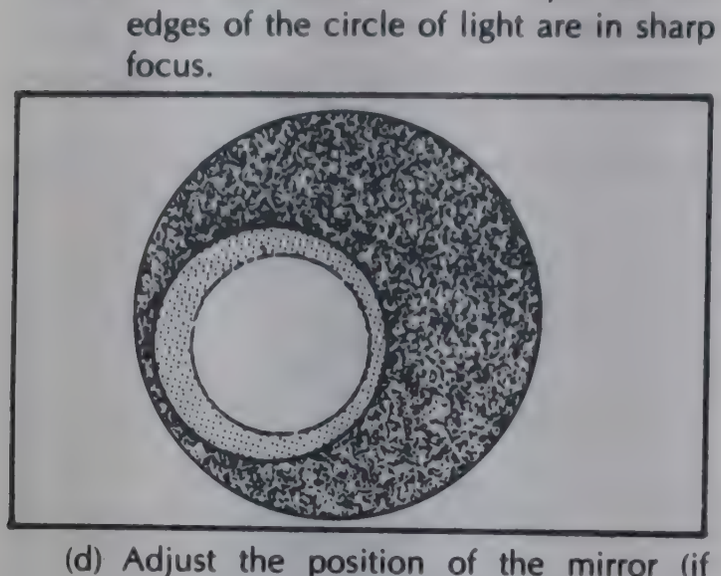


(8) It is very important to centre the condenser correctly:

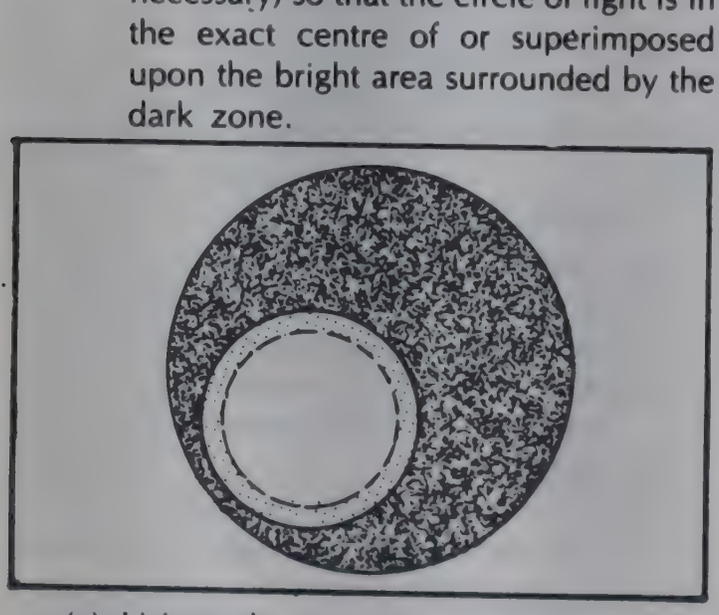
(a) Place a slide preparation without a coverslip on the stage. Lower the condenser. Open the iris diaphragm. Examine with the lowest power objective (× 10). Look through the eyepiece and bring into focus.

(b) Close the diaphragm. A blurred circle of light surrounded by a dark ring appears in the field.

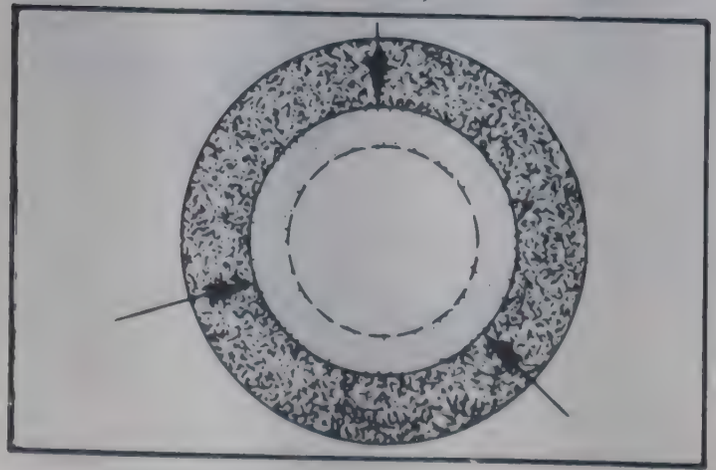
(c) Raise the condenser slowly until the edges of the circle of light are in sharp focus.



(d) Adjust the position of the mirror (if necessary) so that the circle of light is in the exact centre of or superimposed upon the bright area surrounded by the dark zone.



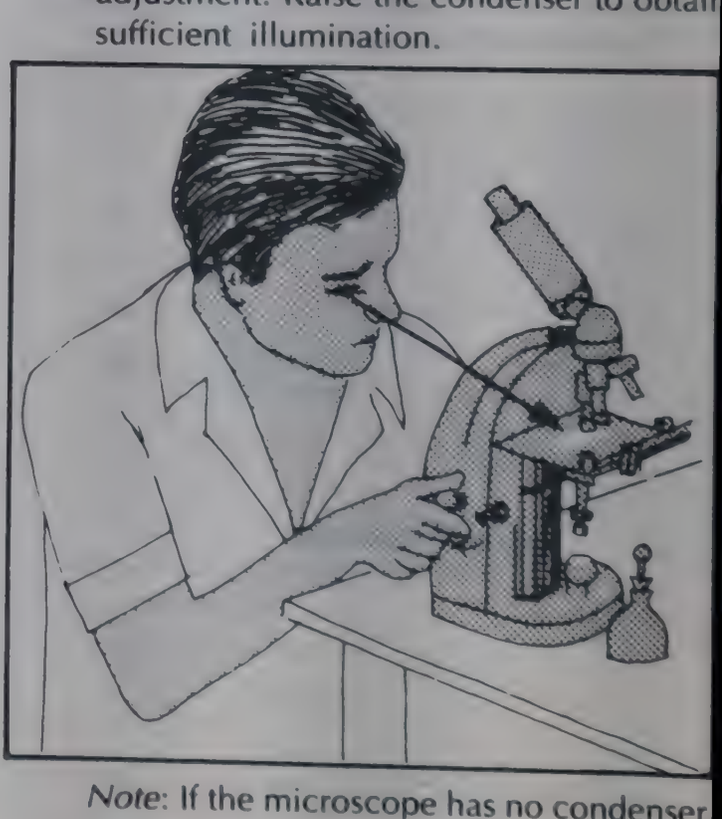
(e) Using the centring screws of the condenser, adjust so that the circle of light is in the exact centre of the field. Check for other objectives also.



(9) Using the low-power objective (× 10): Lower the condenser down to the bottom Lower the objective until it is just abov the slide preparation. Raise the objective using the coarse adjustment screw, until clear image is seen in the eyepiece. (Th working distance is 5 to 6 mm.) Occa sionally a clear image cannot be obtaine although the objective has been lowere as far as possible. This is because the fin adjustment screw has been turned right t the end. Turn it back as far as it will go i the other direction and then focus b raising the objective.

If there is insufficient illumination, rac the condenser up slightly.

(10) Using the high-power objective (× 40): Lower the condenser half-way down Lower the objective until it is just above the slide preparation. (The working dis tance is very short—about 0.5 mm.) Usin the coarse adjustment, raise the objective very slowly until a blurred image appear on the field. Bring into focus using the fine adjustment. Raise the condenser to obtai sufficient illumination.



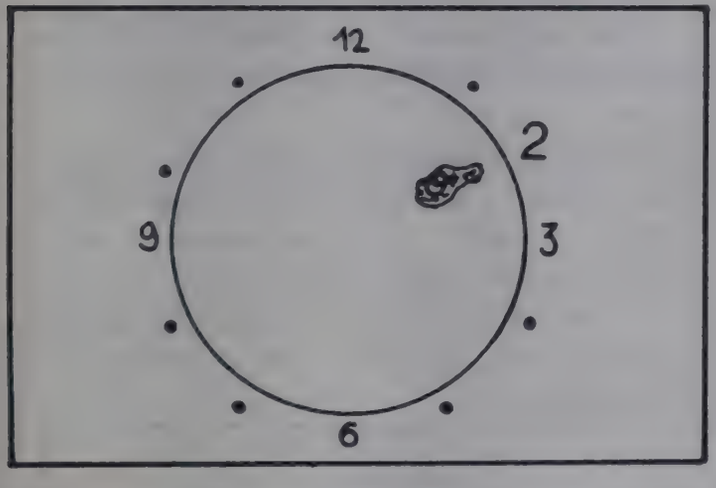
Note: If the microscope has no condenser use the concave side of the mirror.

(11) Using the oil-immersion objective (× 100): Perfectly dry stained preparations must be used. Place a tiny drop of immersion oil on the part to be examined (use synthetic oils, which do not dry, in preference to cedarwood oil, which dries quickly).

Raise the condenser up as far as it will go, and open the iris diaphragm fully. Lower the × 100 objective until it is in contact with the oil. Bring it as close as possible to the slide, but avoid pressing on the preparation. (The working distance is about 0.15 mm.) Look through the eyepiece and turn the fine adjustment very slowly upwards until the image is in focus. If the illumination is inadequate, use the concave side of the mirror.

(12) Remember that the circle of light seen in the eyepiece is called 'the microscopic field'.

The position of objects observed in the field can be described in relation to the hands of a clock. For example, an amoeba is placed at '2 o'clock' in the figure.



(13) *Note:* Objects seen at the bottom of the field are actually at the top. Objects seen on the left hand side of the field are actually on the right. If you move the slide to the right, the object examined moves to the left. If you move the slide towards you, the object examined moves away from you.

2. Routine Maintenance of the Microscope

The microscope needs daily attention to keep it in good working order and thus to ensure reliable laboratory results. Special care is required in hot and humid climates.

(1) Materials required

i. Pieces of soft muslin cloth, e.g. from old, washed cotton sarees
ii. A small bottle of xylene
iii. A plastic cover for the microscope
iv. A small rubber bulb
v. A fine paintbrush
vi. In hot humid climates –
  – Laboratories with electricity: a warm cupboard heated by 1 or 2 light bulbs (40 watt)
  – Laboratories without electricity: a desiccator 15 to 20 cm in diameter with not less than 250 g of dry blue silica gel which indicates humidity by turning pink

(2) Cleaning the objectives

Dry objectives: Breathe on the lens and wipe with a soft cloth, moving the cloth across and not circularly.

Oil-immersion objectives: Remove the oil with a soft cloth. If there are traces of old immersion oil, or if cedarwood oil has been used, moisten the cloth very slightly with xylene and wipe the lens. Then wipe it again with dry cloth.

Every evening before putting the microscope away, remove any dust on the objectives by puffing air with the rubber bulb. If necessary, remove any remaining dust using the fine paintbrush.

(3) Cleaning the eyepiece

Clean the upper surface of the upper lens (where you apply your eye) with a soft cloth.

Clean the lower surface of the lower lens, inside the microscope tube, with a fine paintbrush.

If there is dust inside the eyepiece, unscrew the upper lens and clean the inside lenses using only air from the rubber bulb and the fine paintbrush.

(4) Cleaning the condenser and mirror

The condenser is cleaned in the same way as the objectives, with a soft cloth moistened with xylene. The mirror is cleaned with a soft cloth.

(5) Cleaning the support and stage

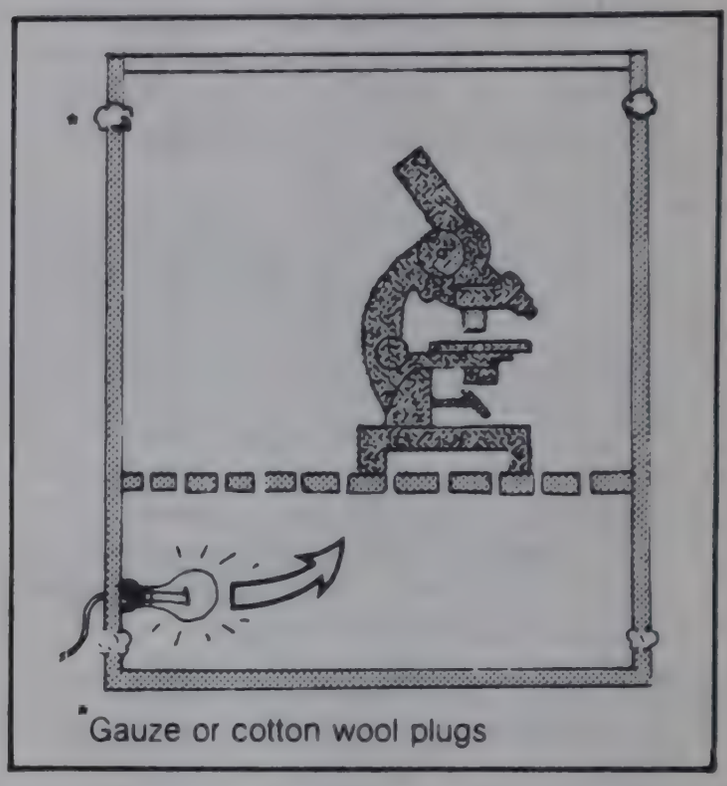
Clean with a soft non-fluffy cloth. Never use xylene, which may remove the black paint from the microscope. The stage can be cleaned thoroughly using a soft cloth.

(6) Additional precautions in hot humid climates

In hot humid climates, if no precautions are taken, fungus may develop on the microscope, particularly on the surface of the lenses, in the grooves of the screws and under the paint, and the instrument will soon be useless. This can be prevented as follows:

Laboratories with electricity:

Every evening place the microscope in a warm cupboard. This is simply a cupboard with a tight fitting door, heated by a 40 watt light bulb. The bulb is left on continuously, even when the microscope is not in the cupboard.



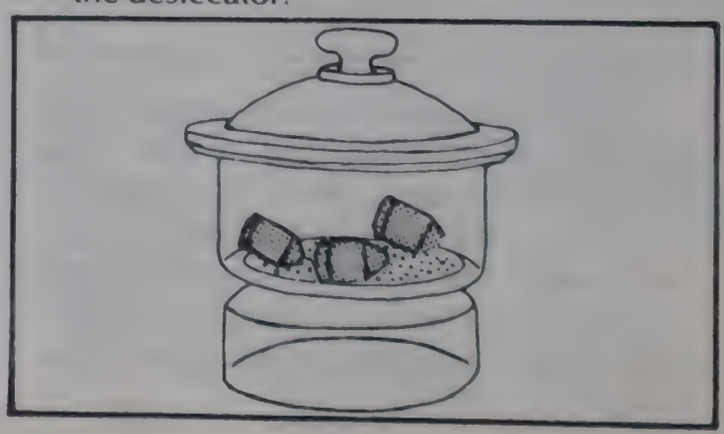
Check that the temperature inside the cupboard is at least 5°C warmer than the temperature of the laboratory.

Laboratories without electricity:

The microscope can always be kept in the open air, in the shade but near a sunny spot.

Never put the microscope in its wooden box (even overnight) but always use a plastic cover. The microscope must, however, be cleaned daily to get rid of dust.

At the end of the day's work, remove the eyepiece and objectives and keep them in the desiccator.



Always keep the openings in the body tube and the nosepiece covered with the caps provided.

(7) Additional precautions in hot dry climates

In hot dry climates the main problem is dust (sandstorms, etc). Fine particles work their way into the threads of the screws and under the lenses. This can be avoided as follows:

i. Always keep the microscope under a plastic cover when not in use. Put it away in its wooden box every evening.
ii. At the end of the day's work, clean the microscope thoroughly by blowing air on it from a rubber bulb.
iii. Finish cleaning the lenses with a fine paintbrush. If dust particles remain on the surface of the objectives, remove with a soft muslin cloth.
iv. If there is a wet season lasting more than a month, take the precautions described above for hot humid climates.

Some things not to do

(1) Never clean the lenses of the objectives and eyepiece with ethanol.

(2) Never dip the objectives in xylene or ethanol. (The lenses would become unstuck.)

(3) Never use ordinary paper, fluffy or rough cloth, or cotton wool to clean the lenses.

(4) Never touch the lenses of the objectives or eyepiece with your fingers.

(5) Never clean the body of the microscope with xylene.

(6) Never clean the *inside* lenses of the eyepiece or objectives with cloth or paper. Use a fine paintbrush only.

(7) Never leave the microscope without the eyepiece or objectives unless the openings are plugged.

(8) Never keep the microscope in a closed wooden box in hot humid climates.

(9) Never put the microscope away with immersion oil on the objective.

(10) Never carry the microscope by the limb with one hand. Use both hands, one under the foot, the other holding the limb.

(11) Never allow any unauthorized person to meddle with the microscope.

Remember: that the microscope is a delicate and costly instrument which must be handled with care. It is your responsibility to see that it is maintained well.

CHAPTER 3

Sterilization

Sterilization means the freeing of any object from living micro-organisms.

You will need to sterilize materials for two main purposes:

(1) In preparation for taking specimens (needles, syringes, tubes, etc. must be sterile)

(2) To disinfect contaminated materials.

Sterilization is carried out either by moist heat, e.g. autoclave, pressure cooker or boiling, or by dry heat, e.g. flaming.

1. The Autoclave

Principle

Water is heated in a closed container.
This produces saturated steam under pressure, with a temperature of over 100°C.

All types of organisms are killed when apparatus is heated for *20 minutes at 120°C* in this steam under pressure.

Components of the autoclave

(1) *Boiler*: A large deep cylinder in which the apparatus to be sterilized is placed.

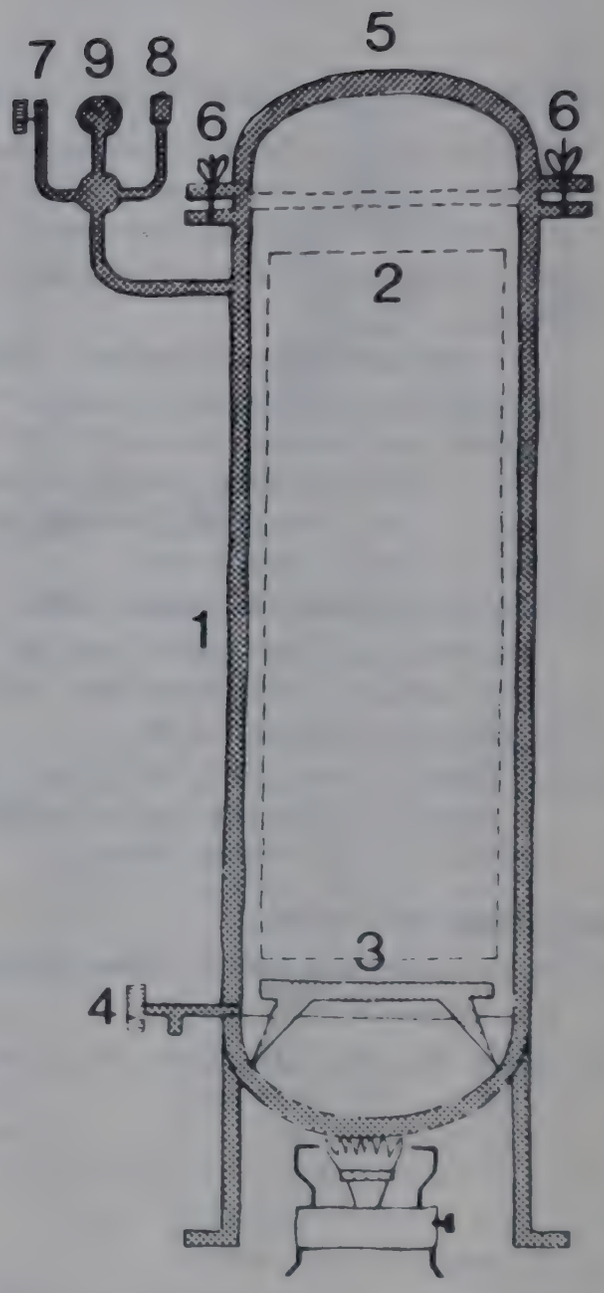
(2) *Basket*: A big wire basket that holds the materials to be sterilized.

(3) *Basket support*: A support in the bottom of the autoclave that holds the basket above the water level.

(4) *Drainage tap*: A tap fitted at the base of the boiler that drains off excess water.

(5) *Lid*: The lid covers and seals the boiler and is fitted with a rubber washer.

(6) *Lid clamps*: These clamps, together with the rubber washer, seal the lid and prevent steam from escaping.



(7) *Air outlet valve*: A valve at the top of the boiler or on the lid used to let air out when the water is first heated.

(8) *Safety valve*: A valve at the top of the boiler or on the lid that lets steam escape if the pressure becomes too high and so prevents an explosion.

9) *Temperature gauge or pressure gauge*: A dial at the top of the boiler or on the lid that shows the pressure, the temperature, or both.

Graduations on the gauge

All gauges indicate the temperature in degrees Celsius (°C). Some also have a second set of figures indicating the pressure.

Heating the autoclave:

The heating system may be built into the instrument in the form of—

- electric elements

or

- gas burners.

Otherwise the autoclave is heated over a Primus stove.

How to use the autoclave

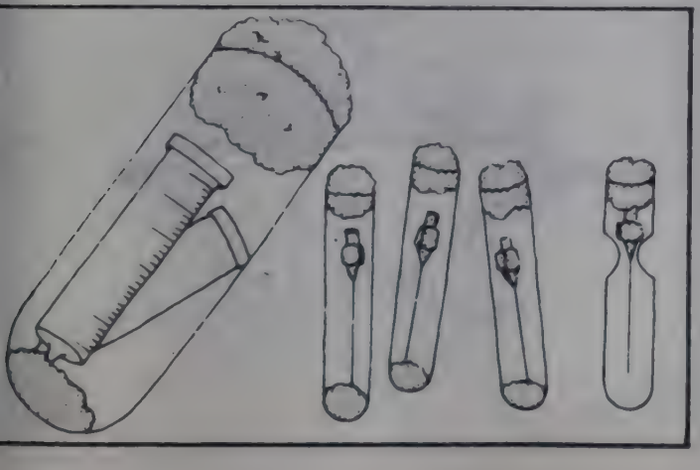
(1) Preparation of material for sterilization:

Syringes: These are placed in large glass tubes plugged with non-absorbent cotton wool (the pistons and barrels are kept separately).

Or

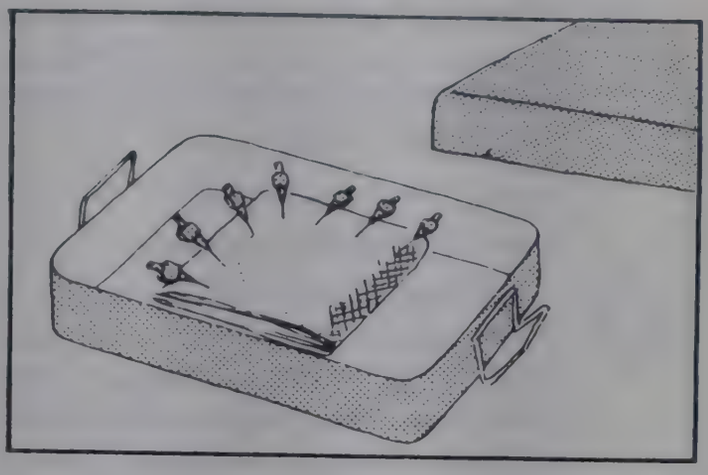
they are wrapped in gauze and placed in metal trays.

Needles: These should preferably be placed separately in small test tubes that are then plugged. Place a pad of non-absorbent cotton wool at the bottom of each tube to protect the tip of the needle.



Or

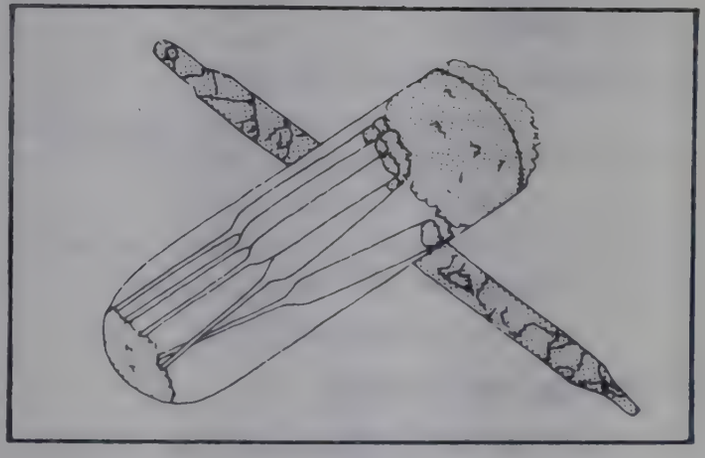
arrange the needles in a metal tray with the points stuck into a folded gauze pad.



The metal trays are placed uncovered in the autoclave.

Glassware: Specimen tubes, Petri dishes, etc. should be wrapped in brown paper and tied with string.

Pasteur pipettes: These are placed in large tubes which are then plugged.



Or

they may be wrapped in several layers of brown paper.

(2) Sterilization procedure:

i. Fill the bottom of the autoclave with water (up to the basket support). Make sure that the water does not touch the basket. If necessary, drain off excess

water by opening the drainage tap.

ii. Put the basket containing the material to be sterilized in the autoclave.

iii. Close the lid, making sure that the rubber washer is in its groove. Screw down the lid clamps evenly and firmly but not too tightly.

iv. Open the air outlet valve.

v. Begin heating the autoclave.

vi. Watch the air outlet valve until a jet of steam appears. Wait 3 or 4 minutes until the jet of steam is uniform and continuous. This shows that all the air has been driven out of the autoclave.

vii. Then close the air outlet valve. Tighten the lid clamps and reduce the heat slightly.

viii. Watch the gauge. When the desired temperature is reached, i.e. 120°C, the heat must be regulated to maintain it. Reduce the heat until the needle on the dial remains at the temperature selected.

ix. Start timing at this point:

- Materials for collecting specimens (syringes, needles, tubes)
  **20 minutes at 120°C*
- Containers of infected material (sputum pot, tubes of pus)
  ** 30 minutes at 120°C*

(3) Turning off the heat:

i. Turn off the heat as soon as the required time is up.

ii. When the temperature falls below 100°C, open the air outlet valve to equalize the pressures inside and outside the autoclave.

iii. When the hissing sound stops, unscrew the lid clamps. Take off the lid. Leave to cool. Then carefully remove the basket of sterile equipment. If drops of water have formed, cover with a clean cloth and let it dry in the sun.

Some things NOT to do

(1) Never touch the drainage tap or outlet valv while heating under pressure.

(2) Never touch the safety valve while heatin under pressure.

(3) Never heat too quickly to bring up th pressure, once the outlet valve is closed

(4) Never leave the autoclave unattended whil the pressure is rising.

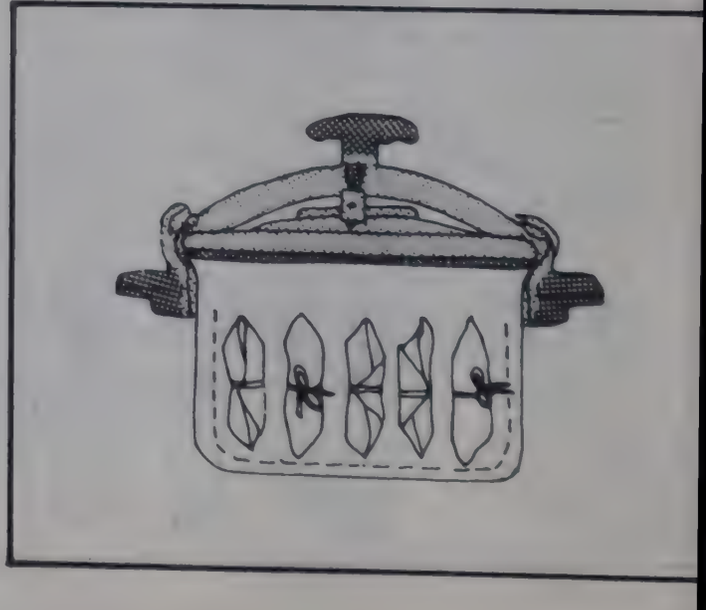
(5) Never leave the autoclave to cool for to long. If it is left for several hours without th overflow valve being opened, a vacuum forms and the sterilized material may break

2. Using a Pressure Cooker

Pressure cookers are large saucepans designed to cook food very quickly, using steam under pressure. They are used in some small laboratories to sterilize specimen collection equipment.

Note: Read the instructions provided with the pressure cooker before using it.

(1) Fill the bottom of the cooker with water Place the material to be sterilized in the basket held above the water level by a support. The wrapped articles should be placed upright. Never lay them flat.



) Fit on the lid and weight according to the instructions provided with the cooker.

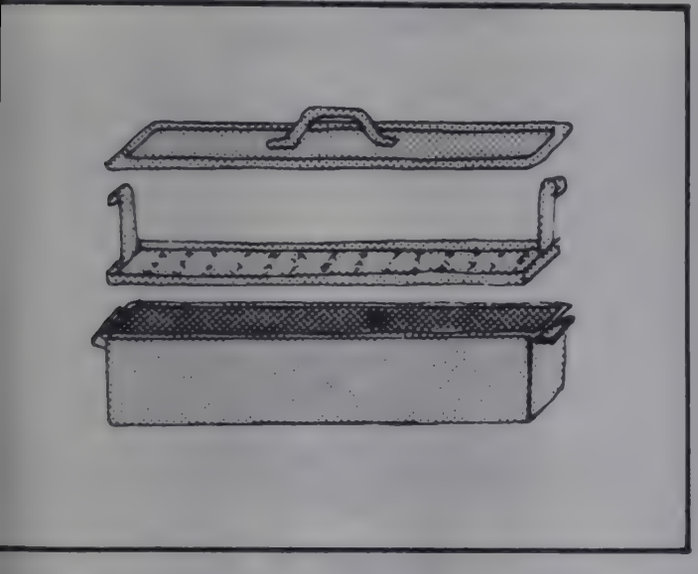
) Place the cooker on the lighted stove.

) When the maximum pressure (15 lb per sq.inch or 1 kg per sq.cm) is reached, lower the heat. Leave on moderate heat for 20 minutes.

) Turn off the heat. Leave to cool or cool under the tap. After the pressure is released, remove the weight and take off the lid. Take out the sterilized material and leave to dry.

. Sterilization by Boiling

his method should be used only where there is o alternative. Use a special boiling pan or, if ot available, a saucepan.



ill the pan with water.

Heat over a stove.

Glass items, e.g. syringes, should be wrapped eparately in cloth and placed in the pan while he water is still cold.

Metal articles, e.g. needles, forceps, scalpels, should be put in when the water is boiling. Allow the articles to boil for 20 minutes.

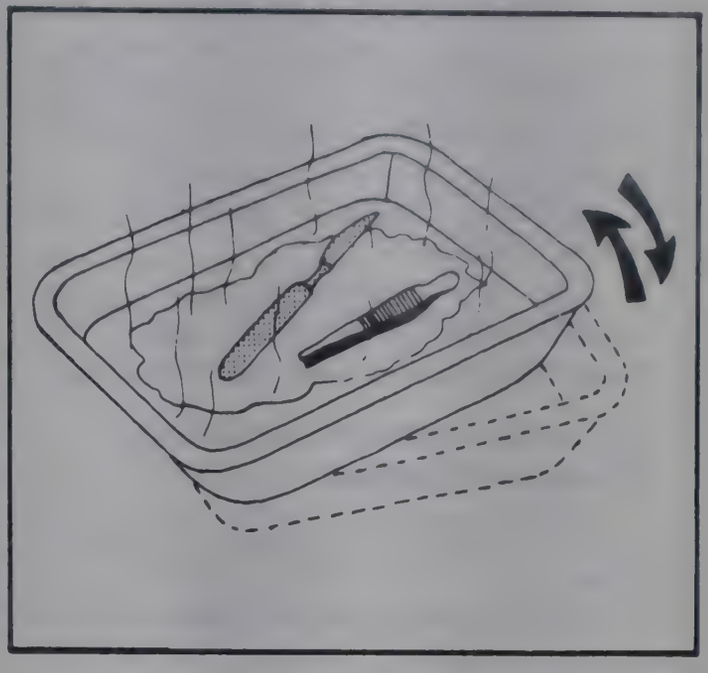
4. Sterilization by Flaming

This is a method that should be used only for metal articles such as forceps and scalpels. It is not suitable for general use.

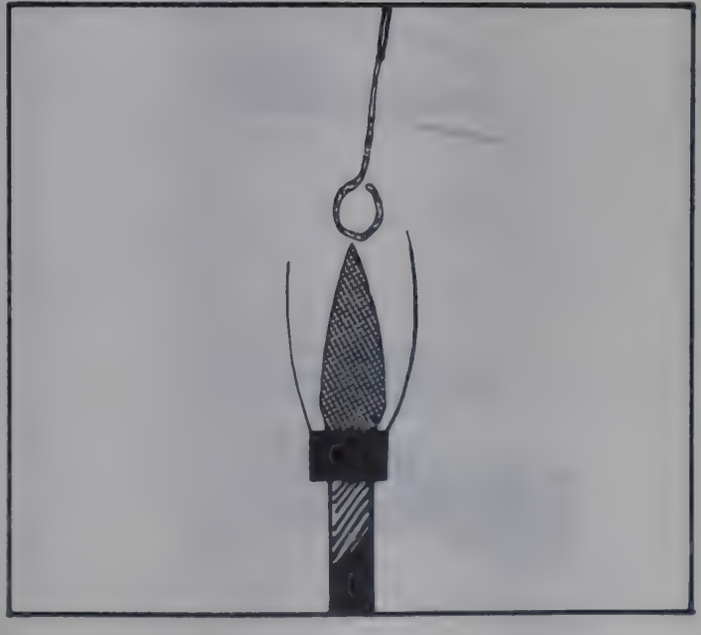
(1) Place the articles in a metal tray.

(2) Add about 10 drops of ethanol and ignite.

(3) Tilt the tray one way, then the other during flaming.



Wire loops, scalpels for taking skin smears, or lancets for taking capillary blood samples should be heated directly in the flame of a Bunsen burner or spirit lamp until they are red hot.



They must be allowed to cool completely before they are used.

CHAPTER 4

Disposal of Specimens and Infected Material

The specimens examined in the laboratory, e.g. stools, pus, sputum, urine, are often infectious. After examination they must be destroyed in such a way that all risk of infection is avoided.

The specimens may be disposed of:

—in cardboard cartons or plastic pots that can be destroyed

—in glass jars and bottles that can be cleaned, sterilized and used again.

All disposable containers are used once only.

1. Disposable Boxes Containing Stools and Sputum

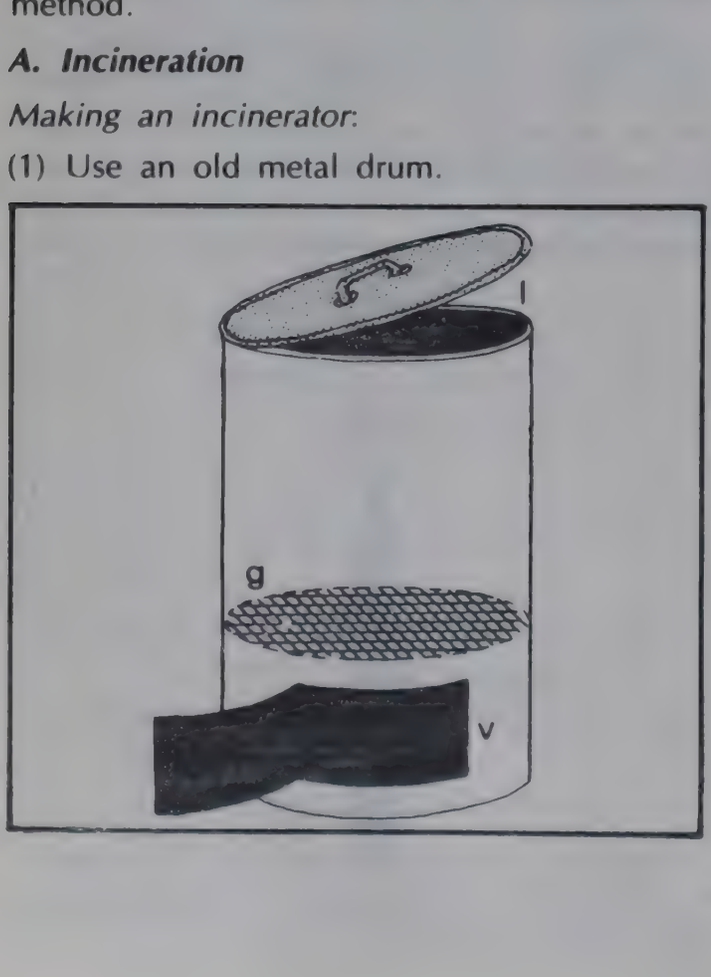
These may be burned (incinerated) or buried in the ground.

Incineration is the easier and more effective method.

A. Incineration

Making an incinerator:

(1) Use an old metal drum.



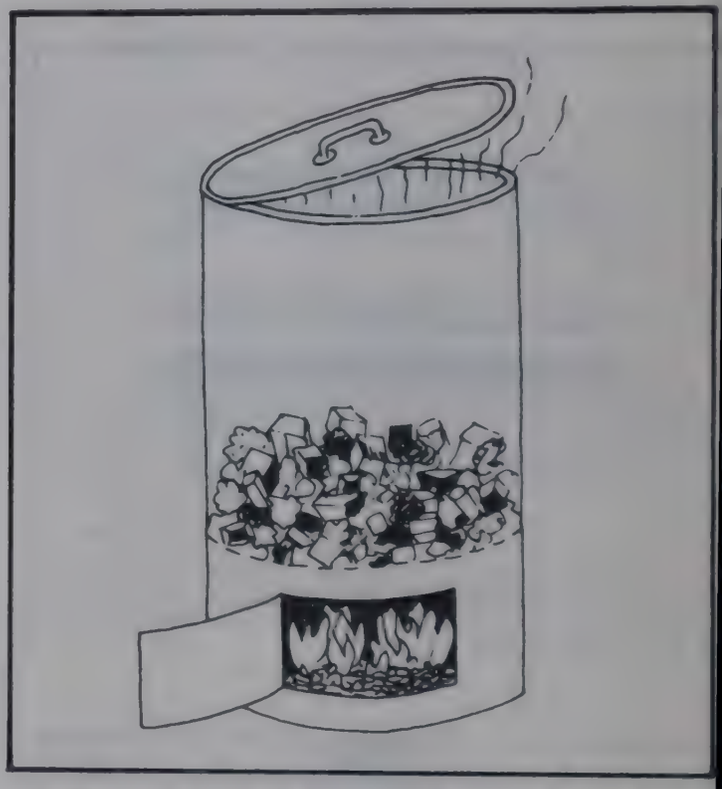
(2) Fix a strong metal grating firmly about one third of the way up the drum (g).

(3) Cut a wide opening or vent below the level of the grating (v).

(4) Find a removable lid for the drum (l).

How to incinerate:

(1) At the end of each morning's and afternoon's work, place all used stool and sputum boxes on the grating of the incinerator.



(2) Always keep the metal drum tightly closed (both lid and vent) except during incineration.

(3) Incinerate once a week, or more often if necessary. Fill the bottom of the drum with paper, sticks, wood shavings, etc.

(4) Remove the lid. Light the fire and keep it burning until all the infected material has been reduced to ashes.

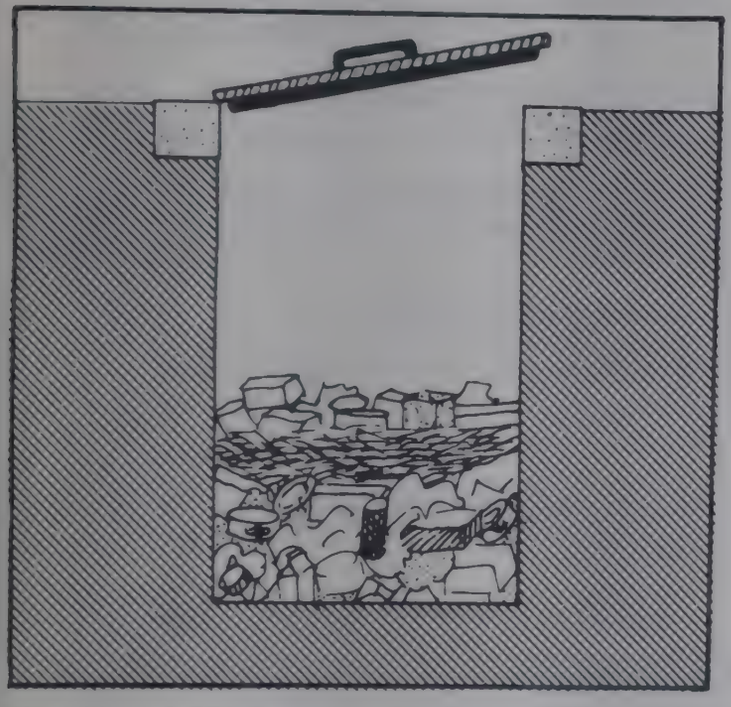
(5) The ash produced is not dangerous and can be thrown on the refuse heap.

B. Burial

(1) Dig a pit 4 to 5 metres deep and 1 to 2 metres wide in a corner of the PHC compound.

(2) Make a lid that fits tightly over the pit. It is advisable to strengthen the upper rim of the pit by lining it with bricks or stones.

(3) Throw stool or sputum boxes and other infected material into the pit twice a day. Replace the lid immediately.



(4) Once a week, cover the refuse with a layer (about 10 cm thick) of dried leaves.

(5) If possible, instead of using dry leaves, add a layer of quicklime (calcium carbonate) once a week.

2. Sterilization and Cleaning of Non-Disposable Containers

This is a more difficult procedure. Hence, where possible, use disposable containers.

The jars and bottles may contain:
- very infectious material, e.g. stools, sputum, pus
- other specimens, e.g. blood, urine.

A. Stool containers

Fill the jars containing stools with a 5% solution of phenol or a similar disinfectant. Leave for 24 hours. Empty into the latrine. If the latrine is connected to a septic tank, phenol or other disinfectant should *not* be added to the stools. Clean the jars with detergent and water, as described in Chapter 1.

B. Sputum pots and tubes of pus

There are several possible methods.

Using an autoclave:

(1) Place the jars in a metal tray and place this in the basket of the autoclave.

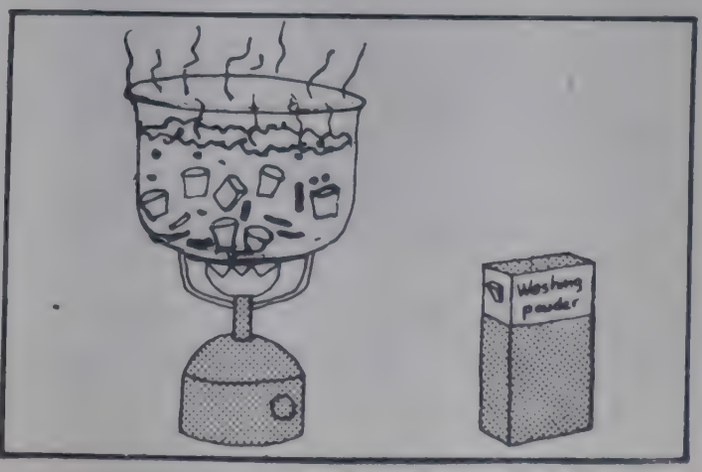
(2) Sterilize for 30 minutes at 120°C to destroy all organisms.

(3) After they have cooled, empty the contents of the jars into the sink or latrine.

(4) Clean the jars with water and detergent.

Boiling in detergent:

Keep a large vessel especially for this purpose. Boil sputum pots:
- for 30 minutes
- in water containing washing powder in a strong solution (60 ml per litre of water)
  Or
  water containing sodium carbonate crystals (washing soda).



Using phenol:

Pour into each sputum pot:
- 5 ml of 5% phenol.

Leave for 24 hours.
Rinse out with water and wash with soap and water.

C. Urine bottles

Empty the bottles into the latrine.
Fill them with:
- a 10% solution of commercial bleaching powder

  Or
- a 2% solution of phenol.

Leave for 24 hours.
Rinse out with water and wash with soap and water.

D. Tubes of blood

Tubes of fresh blood collected the same day should be:
- rinsed in cold water
- left to soak in a detergent solution (See Chapter 1).

Tubes of old blood kept for several days at room temperature where organisms may multiply in them should be:
- filled with a 10% solution of commercial bleaching powder
- left for 12 hours and then rinsed and cleaned with soap and water.

CHAPTER 5

Records and Reports

All specimens must be registered and given numbers when they arrive at the laboratory, and the results of all investigations must be recorded.

This will:

- avoid the risk of getting the specimens mixed up
- make it possible to look up a result
- make the results available for the promotion of public health programmes.

The laboratory should have the following records and report forms:

1. Examination Request Forms that accompany the patient or the specimen
2. A Laboratory Register for recording details concerning the specimen and the results obtained
3. Report Forms for sending the report of the examination carried out to the person requesting it
4. Monthly Report Forms
5. A Stock and Issue Register

1. Examination Request Forms

Each patient on whom an examination is to be carried out should be given an Examination Request Form by the concerned doctor indicating clearly what examination(s) is/are to be carried out. If a specimen is sent to the laboratory for examination, the nature of the specimen and the date and time of taking the specimen should also be indicated on the Examination Request Form. A specimen Examination Request Form is given at Appendix 5.1. This form will also accompany any specimen sent by the PHC to a referral laboratory, e.g. at the Community Health Centre or District Hospital.

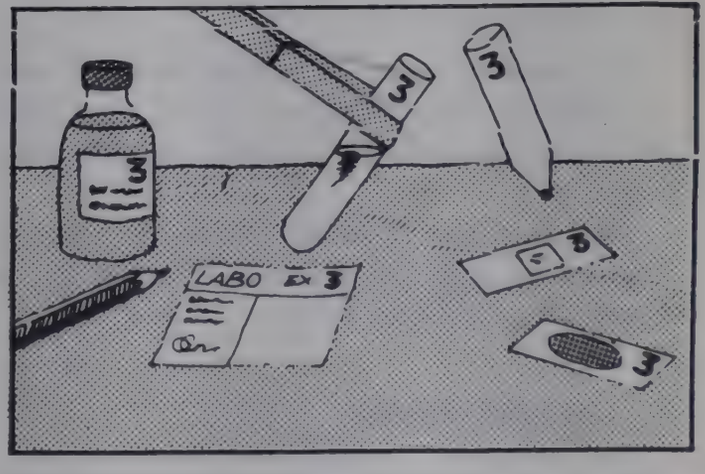
Note: In the case of thick and thin blood smears taken at the periphery for malarial parasites, each smear will be accompanied by two copies of the MIES form 'Report of Blood Smears for Malaria' [See Appendix 5.3C(2)]

All Examination Request Forms received by the laboratory will be filed datewise in an Examination Request File.

Numbering the specimens: Give each specimen a number as soon as it is taken or received. Write this number immediately:

- on the Examination Request Form
- on the specimen container (use grease pencil)
- on every test tube used for the specimen
- on every microscope slide used for the specimen.

This will prevent any mistakes.



Select a convenient method of numbering the specimens. For instance, the specimen numbers

could be in series, starting afresh each year, whatever the specimen received may be, e.g. 1/84 (urine), 2/84 (stool), 3/84 (urine), 4/84 (blood) . . . 376/84 (blood).

Or separate series of numbers can be given to each type of examination, starting afresh each year, e.g.

urine: Ur-1/84, Ur-2/84 . . . Ur-17/84
blood: Bl-1/84, Bl-2/84 . . . Bl-96/84
stool: St-1/84, St-2/84 . . . St-57/84

and so on.

2. Laboratory Register

The Laboratory Register should be a notebook with numbered pages and strong hard covers. Each specimen should be numbered and recorded in the section of the register reserved for that type of specimen.

The Register may be divided into the following 8 sections:

i. Urine analysis
ii. Stool examination
iii. Haematology
iv. Sputum examination
v. Skin and nasal smears
vi. Semen examination
vii. Throat swab collection
viii. Water sampling

Number each page of the Register and make an index for the different sections. Flag each section so that it can be easily found when required.

The suggested columns in the Laboratory Register for each of these tests are given in Appendices 5.2A to 5.2H.

3. Report Forms

Besides being entered in the Laboratory Register, the report of the laboratory investigation should be entered in the relevant Report Form and sent to the doctor requesting the investigation.

The suggested Report Forms for each of these tests are given in Appendices 5.3A to 5.3H.

Note: In the case of thick and thin blood smears received from the subcentres, each specimen will be accompanied by two copies of the MIES form, 'Report of Blood Smears for Malaria' [See Appendix 5.3C(2)]. After examination of the smear, you will fill in the results in these two copies, retain one copy in the laboratory file, and send one copy to the concerned Health Assistant Male.

4. Monthly Report Form

At the end of each month, you will prepare a report of work done during the month and submit it to the MO PHC. A suggested Monthly Report Form is given at Appendix 5.4.

5. Stock and Issue Register

An up-to-date inventory should be maintained by you of all equipment, glassware, chemicals and other materials received and available in stock in the PHC laboratory (See Appendix 5.5).

One page should be used for each article. At the beginning of the Register make an index of all the articles contained in the Register, and indicate the pages at which these articles may be found.

Whenever you indent for supplies of a particular item, note through whom you indent for the item (col. 1), the date of indenting (col. 2), the quantity indented for (col.3) and the balance in stock (col.11). The person indenting for the item will sign in col. 12.

When any fresh supplies are received, enter the date of receipt (col. 4), the stock in hand (col. 5), the quantity received (col.6), the total of cols. 5 & 6 (col. 7) and the balance in stock (col. 11). The person receiving the supplies will sign in col. 12.

Whenever supplies are issued to anyone, or for use in the laboratory itself, or if any article is broken, enter the date of issue (col. 8), the

quantity issued (col. 9), to whom the supply was issued (col.10) and the balance in stock col.11). The person to whom the supplies are issued will sign in col. 12.

A specimen page of this Register might read thus:

Name of Article: Microscope slides (100 slides per packet) *Item No.*: 18

INDENT PLACED			RECEIVED				ISSUED				
Through	Date	Quantity	Dt. of recpt.	Stock in hand	Qnty. recd.	Total	Dt. of issue	Qnty. issued	To whom issued	Balance in stock	Sig.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
MO PHC	2.1.84	6 pkts								3 pkts	LT
			4.2.84	3 pkts	6 pkts	9 pkts				9 pkts	LT
							19.2.84	1 pkt	HWM SC–A	8 pkts	HWM
							22.2.84	1 pkt	Self	7 pkts	LT
							5.3.84	1 pkt	Self	6 pkts	LT
and so on							8.3.84	1 pkt	HWM SC-B	5 pkts	HWM

Appendix 5.1: Examination Request Form

Primary Health Centre:
Subcentre:
Village:

Patient's Name:
Case No:
Main symptoms/signs:
Specimen sent ☐* not sent ☐
*Nature of specimen:
Date & time specimen taken: 19 a.m./p.m
Nature of examination requested:

Signature:
Designation:
Date:

Date & time specimen received by PHC/CHC/District Laboratory: 19 a.m./p.m.
Specimen No:

Signature:
Laboratory Technician
Date:

Appendix 3.2A: Urine Analysis Register

Date	Time recd.	Speci-men No	Patient's name	Sent by	Exam. reqd.	Appear-ance	Sp. Grav.	pH	Glucose	Protein	Bile pig-ments	Bile salts	Ketone bodies	Micr. exam.	Date report sent	Re-marks
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)

Appendix 5.2B: Stool Examination Register

Date	Time recd.	Specimen No.	Patient's name	Sent by	Exam. reqd.	Appearance	Micr. exam.	Date report sent	Remarks
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)

Appendix 31.C: Haematology Register

Date	Time Recd/ by lab	Time Collec-ted in lab	Speci-men No.	Patient's name	Sent by	Exam. reqd.	Hb/ (g/100ml)	Tot RBC (mill/ mm^3)	Tot WBC (No./ mm^3)	Diff. WBC N	E	B	L	M	ESR (mm/hr)	MPs Pf	Pv	Pm	Mixed	MF	Date report sent	Remarks
(1)	(2)		(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)					(11)	(12)				(13)	(14)	(15)

Appendix 5.2D: Sputum Examination Register

Date	Time Recd./Collected by lab	in lab	Specimen No.	Patient's name	Sent by	Exam. reqd.	Appearance	Micr. exam.	Date report sent	Remarks
(1)	(2)		(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)

Appendix 5.2E: Skin and Nasal Smear Register

Date	Time Recd/ Collected by lab	in lab	Specimen Skin / Nasal smear	smear	Specimen No.	Patient's name	Sent by	Exam. reqd.	Micr. exam.	Date report sent	Remarks
(1)	(2)		(3)		(4)	(5)	(6)	(7)	(8)	(9)	(10)

Appendix 5.2F: Semen Examination Register

Date	Time specimen recd.	Specimen No.	Patient's name	Sent by	Exam. reqd.	Volume	Viscosity	pH	Sperm Motility	Sperm Count	Date report sent	Remarks
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)

Appendix 5.2G: Throat Swab Register

Date	Time specimen collected	Specimen No.	Patient's No.	Sent by	Exam. reqd.	Micr. exam. (Gram)	Swab sent for culture Yes/No	Date & time of despatch	Sent to	Remarks
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)

Appendix 5.2H: Water Sampling Register

Sample No.	Date & time sample collected	Date & time sample despatched to Public Health Laboratory	Physical exam.	Free residual chlorine test	Date report sent	Remarks
(1)	(2)	(3)	(4)	(5)	(6)	(7)

Appendix 5.3A: Urine Analysis Report

PHC:

Patient's name: Specimen No.: Date received:
Examination requested by:
Appearance:
Sp. gravity: pH: Glucose: Protein:
Bile pigments: Bile salts: Ketone bodies:
Microscopic examination:
Remarks:

Signature:
Date:

Appendix 5.3B: Stool Report

PHC:

Patient's name: Specimen No.: Date received:
Examination requested by:
Appearance:
Microscopic examination:

Remarks:

Signature:
Date:

Appendix 5.3C (1): Haematology Report

PHC:

Patient's name: Specimen No.: Date received:
Examination requested by:
Hb: g/100 ml
Total WBC: millions/mm^3
Total WBC: /mm^3
Differential WBC: Neutrophils: %
Eosinophils: %
Basophils: %
Lymphocytes: %
Monocytes: %
ESR: mm/hr
Malarial parasites: Present ☐ Pf ☐ Pv ☐ Pm ☐ Mixed ☐ Absent ☐
Microfilariae: Present ☐ Absent ☐
Remarks:

Signature:
Date:

Appendix 5.3C(2): Report of Blood Smears for Malaria

(To be maintained at worker level)

Primary Health Centre ..... Subcentre ..... Date of Despatch .....

Name and Address of the Health Worker .....

Village	No. of the House	Name of Head of the family	Name of the patient	Age	Sex	Sl. No. of Blood Smear	Treatment No. of Tablets given (4 amino)	Date of Collection of Smear	Result				Remarks
									f	v	m	Mixed	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)

Note: This form should be in triplicate and two copies forwarded to the Microscopist of the Primary Health Centre who will retain one copy and send the other to the Health Assistant Male (HAM) after completion.

Date of examination by the Microscopist .....

Signature of Health Worker ..... *Signature of Microscopist* .....

Appendix 5.3D: Sputum Report

Patient's name: Specimen Date received:
Examination requested by:
Appearance:
Microscopic examination:

Remarks:

Signature:
Date:

Appendix 5.3E: Skin & Nasal Smear Report

PHC:

Patient's name: Specimen: Skin ☐ Nasal ☐
Specimen No.: Date received:
Examination requested by:
Microscopic examination:

Remarks:

Signature:
Date:

Appendix 5.3F: Semen Report

PHC:

Patient's name: Specimen No.: Date & time collected:
Date & time received:

Examination requested by:
Volume: Viscosity: pH:
Sperm motility: Sperm count:

Remarks:

Signature:
Date:

Appendix 5.3G: Throat Swab Report

PHC:

Patient's name: Specimen No.:
Date & time collected: Date & time received:
Examination requested by:
Microscopic examination:

Whether swab sent for culture: Yes ☐ No ☐
Remarks:

Signature:
Date:

Appendix 5.3H: Water Sampling Report

PHC:

Sample No.: Date collected: Time collected:
Date examined: Time examined:

Physical examination
Colour:
Turbidity:
Lustre:
Taste or smell:

Free residual chlorine:
Remarks:

Signature:
Date:

Appendix 5.4: Monthly Report for PHC Laboratory

Report for the month of: PHC:

1. *Urine*: No. of specimens examined:

2. *Stool*: No. of specimens examined:
   No. of specimens found positive for: Roundworm:
   Threadworm:
   Hookworm:
   E.histolytica:
   Other parasites:

3. *Blood*: No. of specimens examined:
   No. of examinations carried out: Hb:
   Total RBC:
   Total WBC:
   Differential WBC:
   ESR:

   Blood smears for malarial parasites: smears received:
   smears collected:
   total smears examined:
   No. of smears found positive:

   Blood smears for microfilariae: smears received:
   smears collected:
   total smears examined:
   No. of smears found positive:

4. *Sputum*: smears received:
   smears collected:
   total smears examined:
   No. of smears found positive
   for Mycobacterium tuberculosis:

5. *Skin & Nasal smears*:
   smears received:
   smears collected:
   total smears examined:
   No. of smears found positive
   for Mycobacterium leprae:

Skin	Nasal

6. *Semen*: No. of specimens examined:

7. *Throat swabs*: No. of specimens collected:
   No. of specimens examined (Gram stain):
   No. of specimens despatched for culture:

8. *Water Sampling*:

Signature:
Date:

Appendix 5.5: Stock and Issue Register

Name of Article: Item No:

INDENT PLACED			RECEIVED				ISSUED				
Through	Date	Quantity	Date of receipt	Stock in hand	Quantity received	Total	Date of issue	Quantity issued	To whom issued	Balance in stock	Signature
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)

CHAPTER 6

Safety Measures in the Laboratory

You must remain alert and cautious while working in the laboratory. You must know that careless handling of reagents, glassware or specimens to be tested in the laboratory can cause serious injury and is dangerous to life.

Accidents in the laboratory may be caused by:

(1) Acids } – splashes on the skin
or – splashes in the eye
(2) Alkalis – swallowing

(3) Toxic substances

(4) Heat – open flames
– hot liquids
– inflammable liquids
– explosions

(5) Broken glass

(6) Contamination by infected material

(7) Electric shock

A suggested list of first aid equipment will be found at Appendix 6.1. The items should be readily available in the laboratory. They must not be kept in a locked cupboard.

1. First Aid in Laboratory Accidents

(1) Acid burns

(Nitric, sulphuric, hydrochloric and trichloroacetic acids)

In all cases: Wash immediately with large quantities of water.

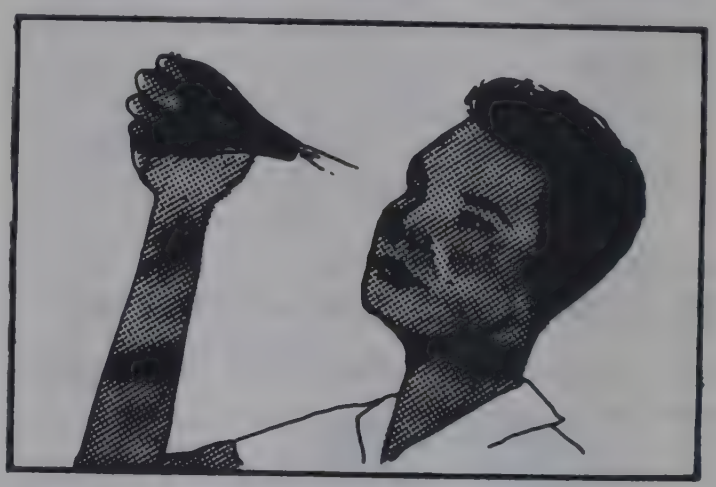
Acid splashes on the skin:

(a) Wash thoroughly and repeatedly with water.

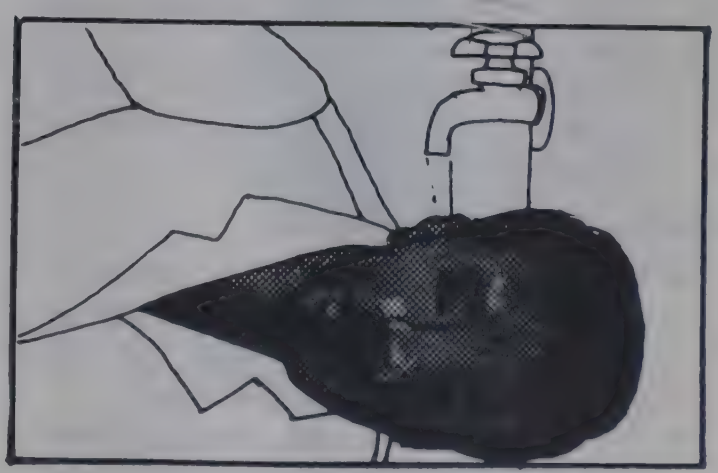
(b) Bathe the affected skin with cotton wool soaked in 5% aqueous sodium carbonate.

Acid splashes in the eye:

(a) Wash the eye immediately with large quantities of water sprayed from a wash bottle or rubber bulb. Squirt the water into the corner of the eye near the nose.



Alternatively, hold the eye under the running tap.



(b) After washing, put 4 drops of 2% aqueous sodium bicarbonate into the eye.
(c) Refer the patient to a physician. Continue to apply bicarbonate solution to the eye while waiting for the doctor.

Swallowing acids:
(accidental swallowing while using a pipette)
(a) Call a physician.
(b) Make the patient drink some 5% soap solution immediately. Alternatively, give him two whites of egg mixed with 500 ml of water or milk. If neither of these is available, he should drink ordinary water.
(c) Make him gargle with the soap solution.
(d) Give him 3 or 4 glasses of ordinary water.
(e) If the lips and tongue are burned by the acid:
- rinse thoroughly with water
- bathe with 2% aqueous sodium bicarbonate.

(2) Alkali burns

(Sodium, potassium and ammonium hydroxide)

In all cases: Wash immediately with large quantities of water.

Important: Alkali burns are as serious as, and often more serious than, acid burns.

Alkali splashes on the skin:
(a) Wash thoroughly and repeatedly with water.
(b) Bathe the affected skin with cotton soaked in 5% acetic acid (or undiluted vinegar).

Alkali splashes in the eye:
(a) Wash immediately with large quantities of water sprayed from a wash bottle or rubber bulb. Squirt the water into the corner of the eye near the nose.
(b) After washing with water, wash the eye with a saturated solution of boric acid (apply drops repeatedly).
(c) Refer the patient to a physician at once.

Swallowing alkalis:
(accidental swallowing while using a pipette)
(a) Send for a physician.
(b) Make the patient drink at once:
– a 5% solution of acetic acid *or* lemon juice *or* dilute vinegar (1 part vinegar to 3 parts water).
(c) Make him gargle with the same acid solution.
(d) Give him 3 or 4 glasses of ordinary water.
(e) If the lips and tongue are burned by the alkali:
- rinse thoroughly with water
- bathe with 5% acetic acid.

(3) Poisoning

This can be caused by:
– inhaling toxic vapours or gases (e.g. chloroform)
– accidental swallowing while pipetting a poisonous solution.

In all cases:
(a) Send for a physician or qualified nurse, specifying the toxic substance involved.
(b) Place the victim in the open air while waiting for the physician.

(4) Burns caused by heat

They fall into two categories:
– Severe burns: affecting large areas of skin, e.g. burns caused when burning ether or boiling water is spilled over the victim.
– Minor burns: affecting a small area of skin, e.g. burns caused by hot glassware or a Bunsen flame.

Severe burns:
(a) If the victim is on fire, e.g. if splashed with burning ether or other inflammable solvent, roll him in a blanket or overall to smother the flames.
(b) Inform the physician on duty immediately.
(c) Lay the victim on the ground. Do not remove his clothing. Cover him if he is cold.

(d) Do not apply any treatment to the burns. This must be left to the physician.

Minor burns:

(a) Plunge the affected part into cold water or ice-water to soothe the pain.
(b) Apply mercurochrome or acriflavine ointment to the burn.
(c) Apply a dry gauze dressing loosely.
(d) If the burn becomes infected or does not heal, refer the patient to a physician.

Note: Never tear off the blisters that form over the burns.

(5) Injuries caused by broken glass

These are caused by broken test tubes, syringes or other glassware.

(a) Wash the wound immediately to remove any glass pieces.
(b) Apply mercurochrome or acriflavine ointment to the wound.
(c) Cover with gauze and adhesive tape.
(d) If the cut bleeds profusely, stop the bleeding by pressing down on it with a compress. Refer the patient to a physician.
(e) If the cut bleeds heavily with the blood spurting out at intervals, try to stop the bleeding with a compress and call a physician or qualified nurse.
(f) Continue to press on the wound while awaiting the physician's or nurse's arrival. He or she will decide whether a tourniquet should be applied.

(6) Contamination by infected material

Wounds caused by broken glassware containing stools, pus, etc.:

(a) Wash the wound immediately.
(b) Check whether the cut is bleeding. If not, squeeze hard to make it bleed for several minutes.
(c) Bathe the whole area, i.e. the edges of the cut and inside the cut, with antiseptic lotion.
(d) Wash thoroughly with soapy water.
(e) Bathe again with antiseptic lotion.
(f) Refer the patient to a physician if the material involved is known to be very infective, e.g. pus.

If infected material is accidentally sucked into the mouth:

(a) Spit it out immediately.
(b) Wash out the mouth with diluted antiseptic lotion.
(c) Wash out the mouth thoroughly with large amounts of clean water.

(7) Bodily damage by electric shock

A low-voltage alternating electric current (220 V) is usually used in the laboratory and electric shocks are rare. They may occur when faulty equipment is being handled, particularly with wet hands. The symptoms are fainting and asphyxia.

(a) Before doing anything else, put off the main switch.
(b) Send for a physician.
(c) Begin giving mouth-to-mouth respiration immediately.

2. Precautions for the Avoidance of Accidents

(1) Handling acids and alkalis:

(a) Diluting sulphuric acid with water: Always add the sulphuric acid to the water drop by drop, stirring the mixture after

each drop. Do this preferably in a sink. *Never pour water into sulphuric acid* (because of the danger of splashing).

(b) Bottles of acids and alkalis: Keep them on the lower shelves of the cupboards. When you take one out, hold it firmly upright with a dry hand. Do not keep acids and alkalis in bottles with ground glass stoppers as they may get stuck.

(c) Pipetting: Where possible, use small measuring cylinders for measuring acids and alkalis. If more accurate measurement is required, use a pipette plugged with non-absorbent cotton wool or with a rubber tube attached. Pipette slowly, watching the level of the liquid.

(2) Heating glassware and liquids

(a) Test tubes: Never heat the bottom of a test tube. The liquid inside might sputter. Heat the middle of the tube, shaking gently. The mouth of the tube should be facing away from the worker and any other person, towards an empty space or a sink.

(b) Ordinary glass and Pyrex: Only Pyrex glassware and porcelain receptacles can be heated over a Bunsen flame. Ordinary glass will break.

(c) Inflammable liquids: Only small quantities of inflammable liquids such as ether, ethanol, acetone, benzene, toluene and carbon disulphide should be kept in the laboratory.

Warning: Ether will ignite at a distance of several metres from a flame. Never place a bottle of ether on a workbench where there is an open flame (Bunsen burner, spirit lamp, etc.). Carbon disulphide is even more dangerous.

(d) Butane gas: When lighting a gas burner, always light the match and hold it to the burner before turning on the gas tap. Turn off the main valves of all butane gas cylinders every evening. Replace the rubber connecting pipes once a year.

(3) Do not use broken, cracked or chipped laboratory glassware.

(4) Put clear labels on poisons. Keep them in a locked cupboard.

(5) Do not use nylon clothes while working as these are easily inflammable. Always use a laboratory apron.

(6) Always ensure that electrical wiring and electrical appliances are in good condition.

Appendix 6.1 Suggested List of First Aid Equipment for PHC Laboratory

1. 5% aqueous sodium carbonate
2. 2% aqueous sodium bicarbonate in an eyedrop bottle
3. 5% acetic acid
4. Saturated solution of boric acid in an eyedrop bottle
5. Soap powder solution (5 g per litre of water)
6. Acriflavine ointment
7. Mercurochrome 2%
8. Antiseptic lotion
9. Cotton wool
10. Gauze
11. Roller bandage
12. Adhesive tape
13. Scissors

PART II

SPECIAL LABORATORY PROCEDURES

CHAPTER 7

Examination of Urine

. Collection of Urine Specimen

rine specimens must be collected:
– in the correct way
– in suitable containers.
If the specimen is not collected properly, the boratory findings will be unreliable.

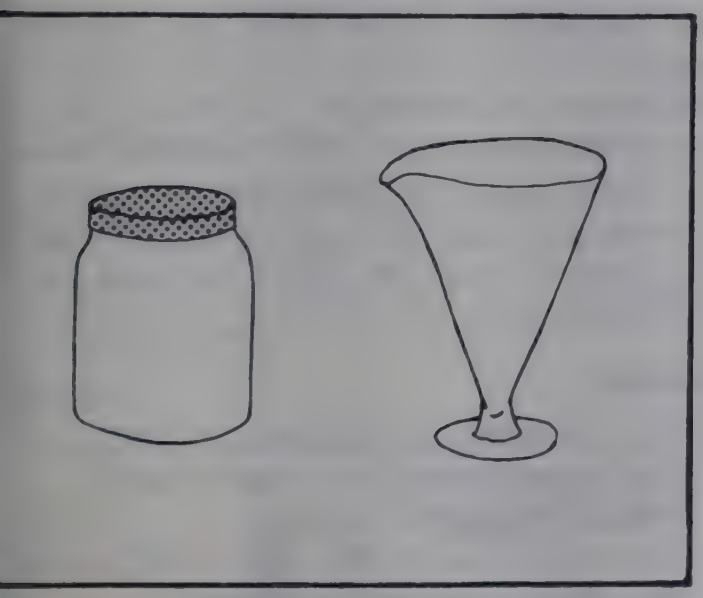
1) Time of collection

Where only one specimen is needed, the best me to collect it is first thing in the morning (the rine is concentrated).

Or have the patient pass the specimen at the HC laboratory.

2) Specimen containers

or collecting urine use:
– a clean wide-mouthed bottle with a cover;
or
– a clean conical urine jar.



3) Quantity

Collect at least 50 ml of urine.

(4) Method of collection

A mid-stream specimen is usually collected as follows:

The patient passes a small amount of urine into the latrine and stops passing urine. He then collects the next part of the urine in the container and stops just before finishing. He then passes the rest of the urine into the latrine.

Women patients should be instructed to wash the genital area before collecting urine. Avoid collecting urine specimens during the menstrual period.

In infants, urine can be collected into a plastic bag with an adhesive mouth. The bag is fixed around the baby's genitalia and left in place for 1 to 3 hours.

2. Appearance of Urine

Observe and describe the appearance of the urine specimen. It may be:
– colourless, yellow, dark yellow, brown (like tea without milk), or bloodstained
– clear or cloudy.

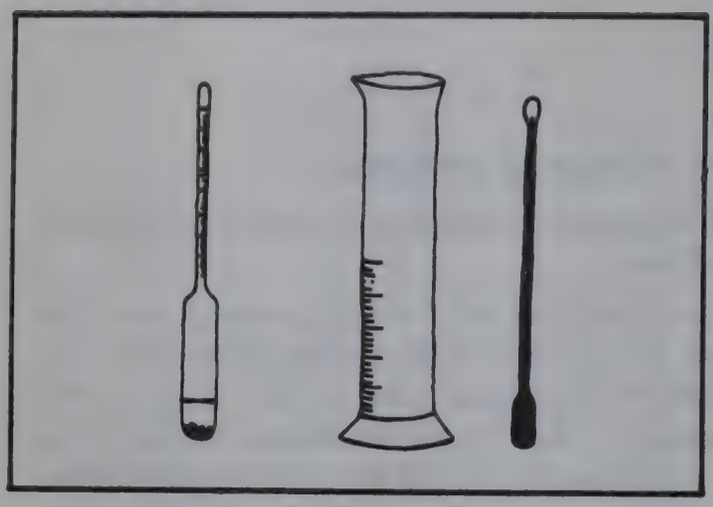
3. Specific Gravity (SG)

Specific gravity is measured by means of a urinometer calibrated from 1.000 to 1.060. (The specific gravity of distilled water is 1.000 at a temperature of 20°C).

The temperature of the urine must also be measured for correct calculation of the specific gravity.

The specific gravity of urine varies according to kidney function.
– Concentrated urine = high SG.
– Dilute urine = low SG.

Materials



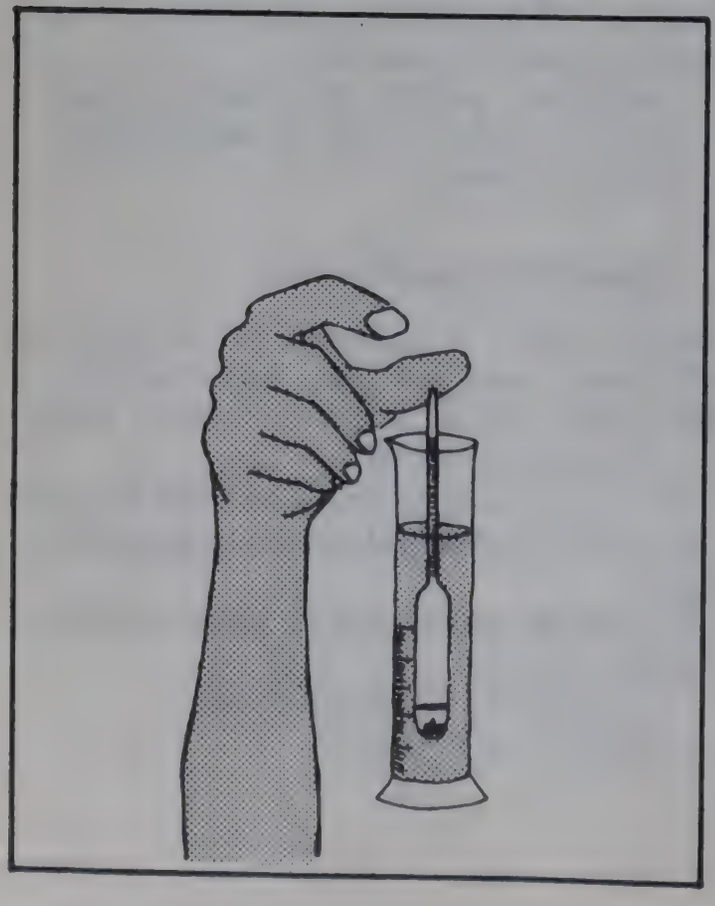
– 1 urinometer
– 1 thermometer (0–50°C)
– 1 measuring cylinder (50 ml)

At least 40 ml of urine are required.

Method

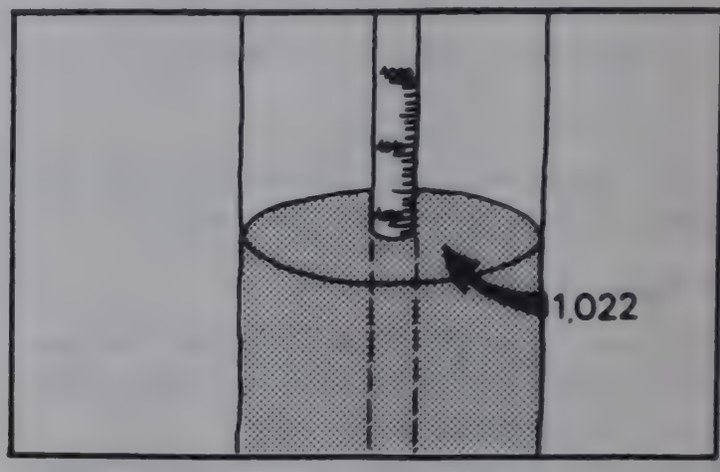
(1) Pour about 40 ml of urine into the cylinder.

(2) Lower the urinometer gently into the urine and release.



(3) Wait for it to settle. It must not be in contac with the sides or bottom of the cylinder.

(4) Read off the SG given on the scale at the surface of the urine (lowest point of the meniscus).



(5) Remove the urinometer. Take the temperature of the urine at once with the thermometer.

Calculation

Check the temperature at which the urinometer is calibrated (marked on the instrument by the manufacturer). It is usually 20°C.

The temperature of the urine has been recorded.

Add to the SG recorded:

– 0.001 for every 3°C that the urine temperature is above the calibration temperature.

Alternatively, subtract from the SG recorded:

– 0.001 for every 3°C below the calibration temperature.

Example

The urinometer is calibrated at 20°C.
The temperature of the urine is 26°C.
The SG measured is 1.021.
The temperature of the urine is 6°C higher than the calibration temperature.
Therefore, add to the SG figure:

$$\frac{6}{3} \times 0.001 = 2 \times 0.001 = 0.002$$

The actual SG of the urine is, therefore:
1.021 + 0.002 = 1.023

esults

ormal SG: 1.020 (normal range: 1.010 – 1.025)
ow SG : below 1.010* (kidney or endocrine disorder)
igh SG : above 1.025 (glycosuria, proteinuria)

Note: A low figure is of no significance if the atient has drunk a large amount of liquid efore the test.

hecking the urinometer

very 3 months check the accuracy of the rinometer in distilled water at the temperature calibration. The reading should be 1.000.

. Measurement of pH

ormal freshly passed urine is slightly acid, ith a pH of around 6.0. In certain diseases the H of the urine may increase or decrease.

rinciple

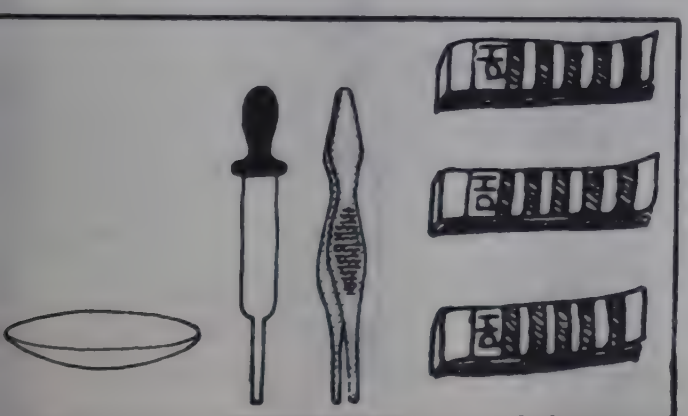
oloured indicator papers are dipped in the rine.

he colour changes according to the pH.

he papers are then compared with a standard ontrol chart giving the corresponding figures.

eminder: Acid fluids have a pH of 0 – 7) being the most acid).
lkaline fluids have a pH of 7 – 14 (14 being e most alkaline).

laterials

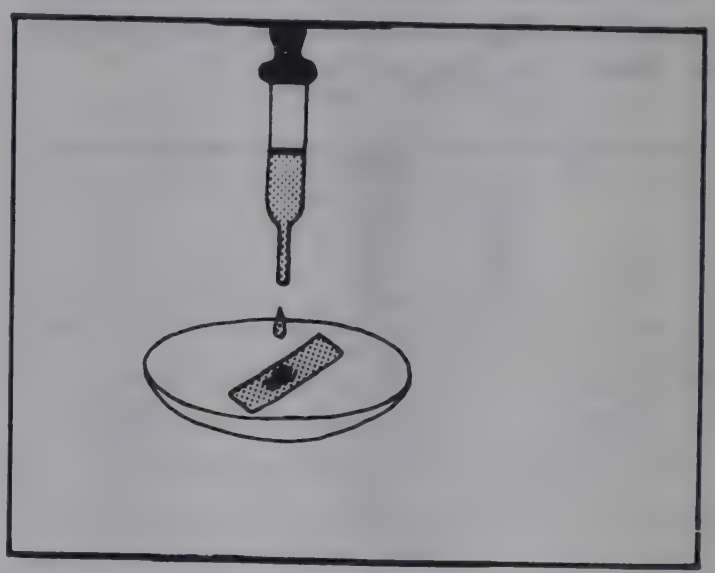


– Watch glasses
– Dropper
– Forceps
– Universal indicator papers (for measuring pH from 1 to 10)
– Indicator papers of limited pH range: for the 5.0–7.0 range and for the 6.0–8.0 range.

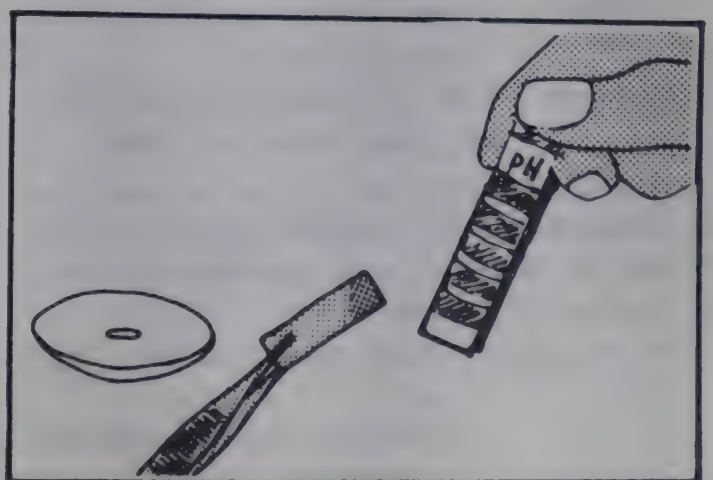
The urine tested must be *fresh*.

Method

(1) Place in a watch glass 1 strip of universal indicator paper (pH 1–10). Let a few drops of fresh urine fall on to the paper.



(2) Pick up the strip of paper with forceps. Compare the colour obtained with those shown on the standard chart. Read off the pH unit given for the colour most closely matching the test paper.



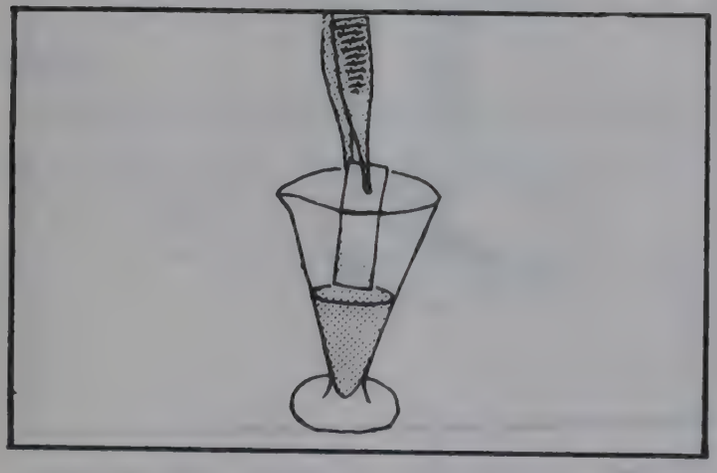
(3) According to the result obtained, select a strip of indicator paper for the corresponding limited range.

Example:

- pH 6 : indicator paper for the range 5.0--7.0
- pH 8 : indicator paper for the range 6.0– 8.0

(4) Repeat the test in another watch glass, using the limited-range paper.
Read off the pH of the urine on the standard chart.

Note: The test paper can also be dipped directly into the urine in the specimen container to obtain a reading of the pH.



Results

Normal pH : about 6.0 (limit of normal range from 5.0 to 7.0 during the day).

Acid pH : 4.5–5.5 (if persistent: some forms of diabetes, muscular fatigue, acidosis).

Alkaline pH : 7.8–8.0 (infections of the urinary tract, vegetarian diet).

Determination of the pH of urine is useful for the identification of crystalline deposits. Some crystals are deposited in acid urine only others in alkaline urine only.

For example: – Acid urine: oxalates, uric acid
– Alkaline urine: phosphates, carbonates.

5. Detection of Glucose in Urine

Principle

Glucose (sugar found in the urine of diabetics) is a reducing substance. It reduces the *blue* copper sulphate of Benedict solution to *red* copper oxide, which is insoluble.

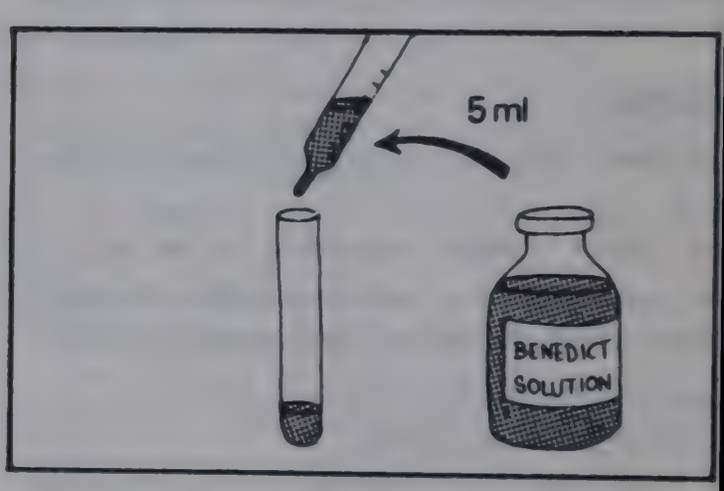
Benedict method

Materials

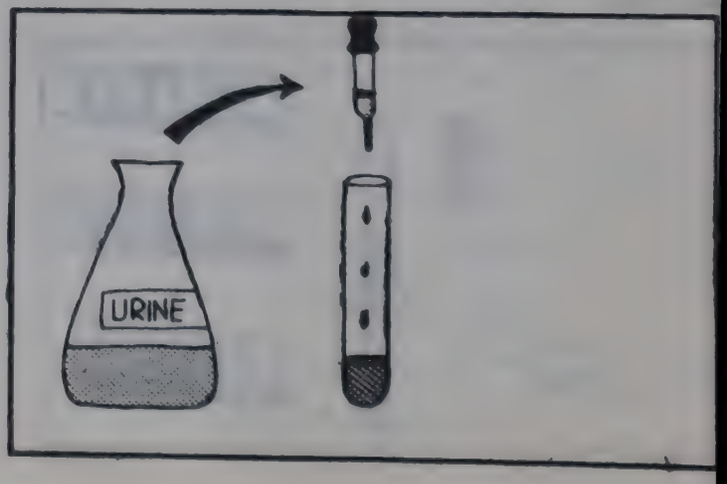
- Pyrex test tubes
- Test tube holder
- Bunsen burner or spirit lamp
- Pipettes
- Benedict qualitative solution (Reagent No. 4)

Method

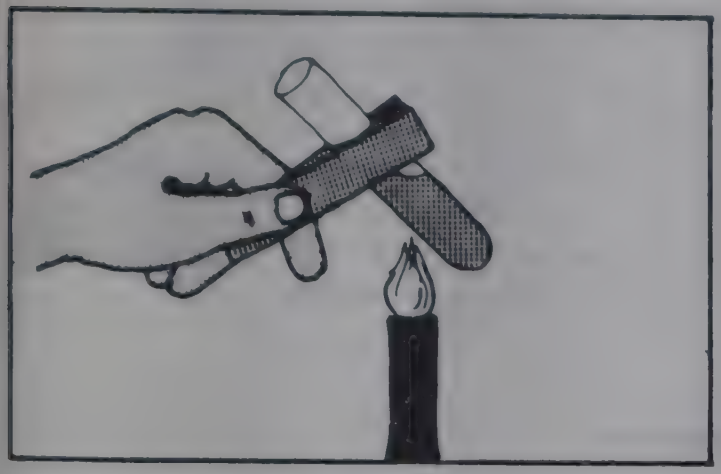
(1) Pipette 5 ml of Benedict solution into a test tube.



(2) Add 8 drops of urine and mix well.



(3) Boil over a Bunsen burner or spirit lamp for 2 minutes.



(4) Leave the mixture to cool to room temperature,

Reading the result

Examine the mixture for any colour change and for precipitate:

Colour	*Result (glucose present)*
Blue	Negative
Green	A trace
Green with yellow precipitate	+
Yellow to dark green	++
Brown	+++
Orange to brick red	++++

Note: For detecting sugar in urine using reagent strips, see Section 7.

6. Detection of Protein in Urine

When urine containing protein is boiled, a cloudy layer is formed which does not disappear on adding acetic acid.

Materials

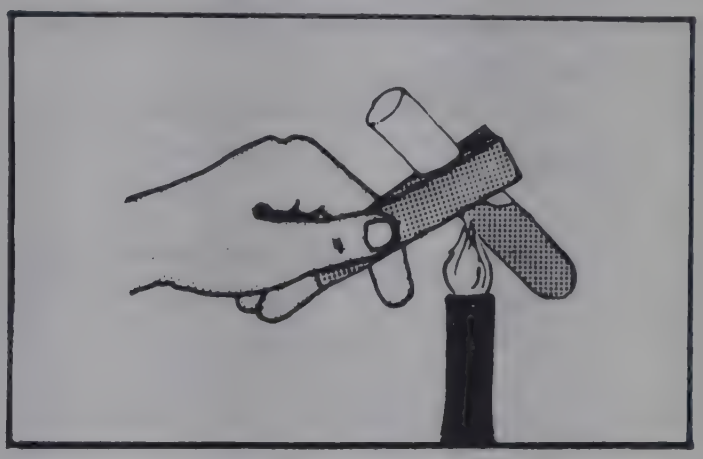
- Pyrex test tubes
- Test tube holder
- Bunsen burner or spirit lamp
- Pipette
- 5% Acetic acid (Reagent No. 1)

The urine must be clear. If it is cloudy, filter it through filter paper, or use the supernatant fluid from a centrifuged urine specimen.

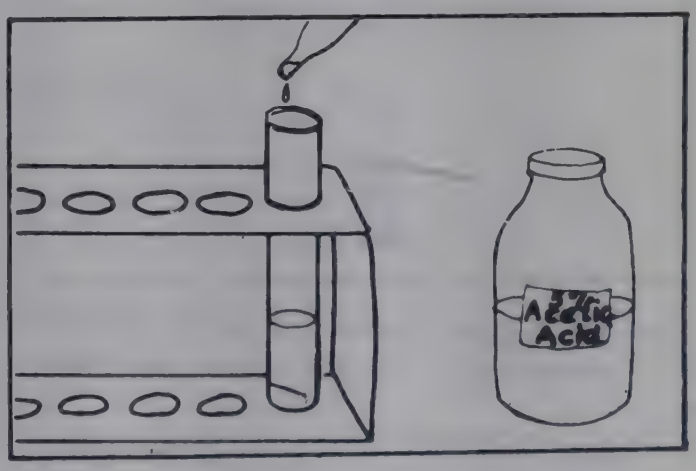
Method

(1) Fill the test tube about two thirds full of urine.

(2) Hold the test tube at an angle to the flame so that the upper layer of urine is brought to the boil.



(3) If the urine becomes cloudy, add a few drops of acetic acid to it. Normally the cloudiness will disappear when this is done.



(4) Bring the urine to the boil again. If the cloudiness persists, it means that protein (albumen) is present.

Results

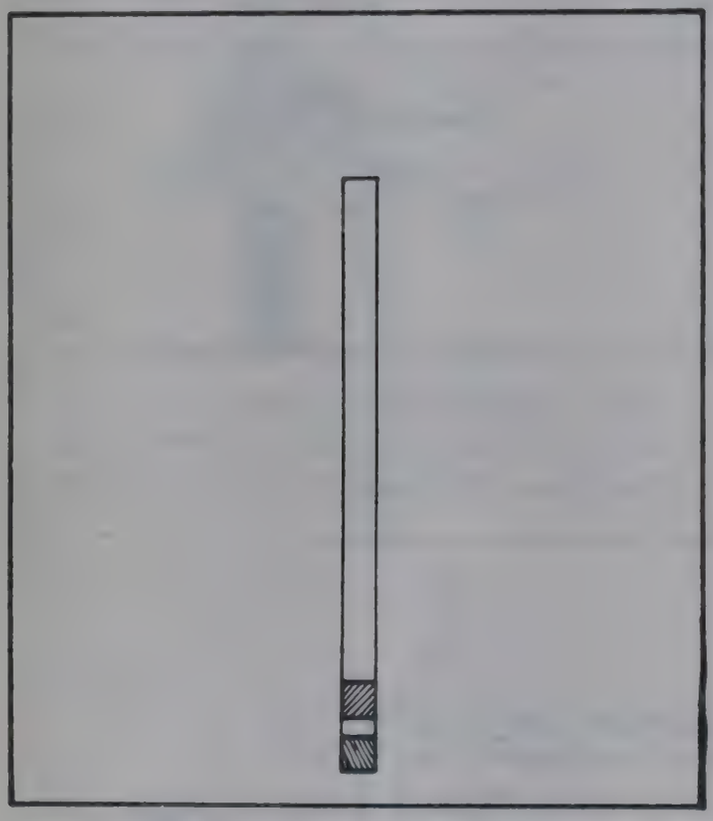
No cloudiness : Negative

Slightly cloudy : Trace of albumen
Heavy cloudiness : Positive for albumen

Note: For the detection of protein in urine using reagent strips, see Section 7.

7. Use of Reagent Strips for Testing Urine for Glucose and Albumen

A simple method of testing urine for the presence of sugar and protein is the use of special reagent strips (URISTIX). These are plastic strips packed in bottles. Each strip has at one end two separate test areas, one for glucose and one for albumen.



The following precautions should be taken to avoid deterioration of the strips and false results.

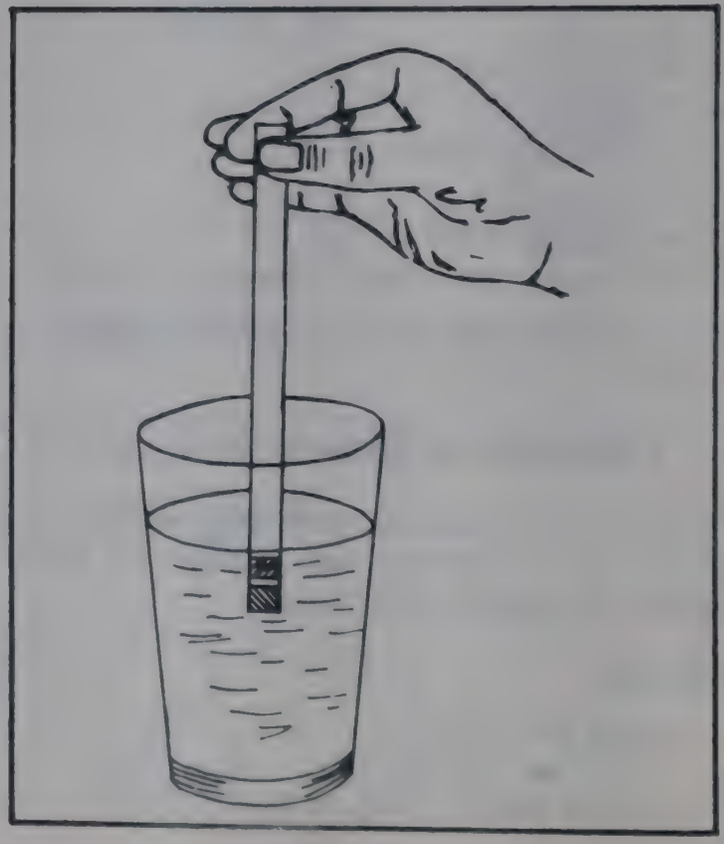
i. Always keep the cap of the bottle tightly closed.
ii. Store in a cool dry place. Do not store in a refrigerator.
iii. Do not open the bottle in a hot steamy atmosphere. Avoid exposing the strips to moisture, direct sunlight, heat, fumes or detergents.
iv. Remove only one reagent strip at a time from the bottle. Do not touch the test areas of the strip. After removing a strip, replace the cap immediately and tightly.
v. Leave the unused strips in the original bottle with the desiccant
Do not transfer them to another container.
vi. Use each strip only once.
vii. Do not use the strips after the expiry date marked on the container.

Materials

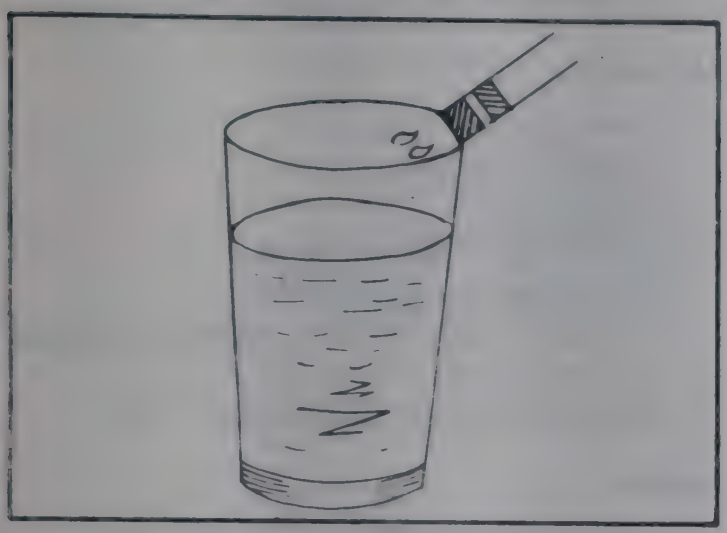
– Reagent strips (URISTIX)
– Fresh urine specimen

Method

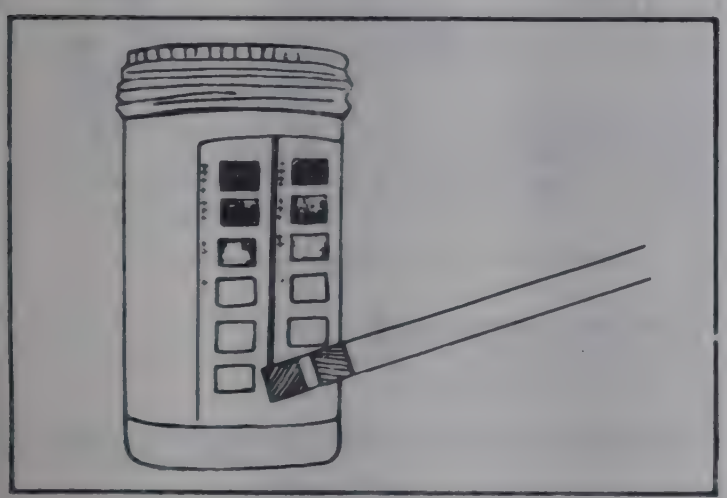
(1) Collect the urine in a clean container and test it as soon as possible after collection.
(2) Carry out the test in good light.
(3) Dip the test areas of the strip in the urine specimen for 1 to 2 seconds only.



(4) Remove the strip and gently tap the edge of the strip against the rim of the urine container to remove excess urine.



(5) Exactly 30 seconds after removing the strip from the urine, hold the glucose test area close to the corresponding glucose colour chart on the bottle label. Compare the colour of the test area of the strip to the closest matching colour on the colour chart and read the results according to the colour chart.



(6) Hold the albumen test area close to the corresponding protein colour chart on the bottle label. Compare the colour of the test area of the strip to the closest matching colour on the colour chart and read the results according to the colour chart. The albumen test area may be read any time up to one minute after dipping in the urine specimen.

Results

Glucose: Negative (blue)
Trace
+
++
+++
++++ (brown)

Albumen: Negative (Yellow)
Trace
+
++
+++
++++ (green-blue)

8. Detection of Bile Pigments in Urine

The bile secreted by the liver contains greenish-yellow substances—bile pignents. In certain liver diseases (jaundice), anaemias and infectious conditions, bile pigments may pass into the blood stream and be excreted in the urine.

A. Lugol iodine test

Principle

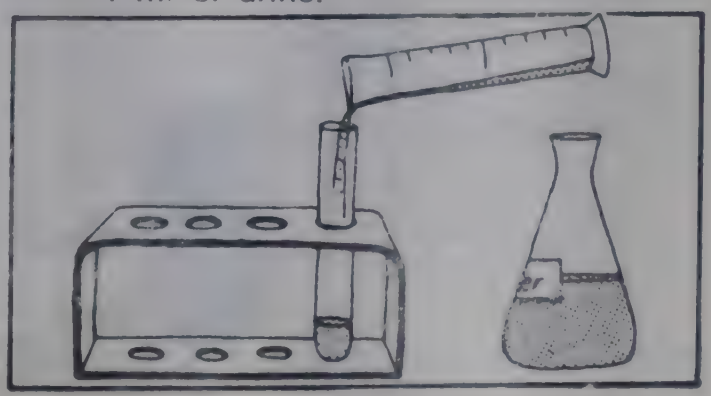
When iodine is added to urine containing bile pigments, a green colour is produced.

Materials

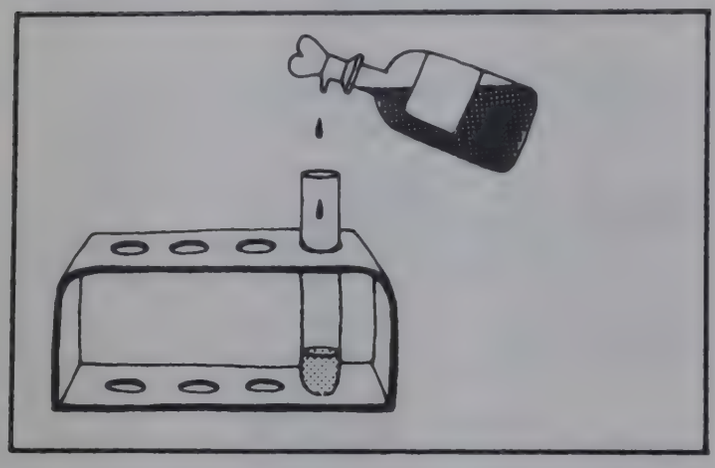
- Test tubes and rack
- 10 ml measuring cylinder
- Lugol iodine solution (Reagent No. 19)
- Dropping pipette

Method

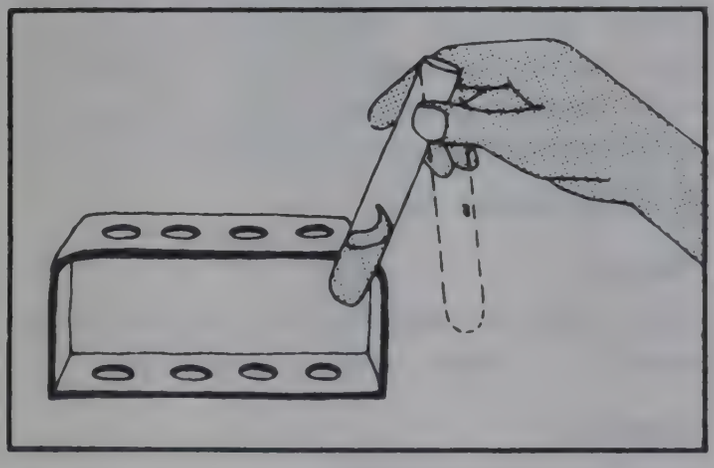
(1) Pour into a test tube:
- 4 ml of urine.



(2) Add:
– 4 drops of Lugol iodine solution.

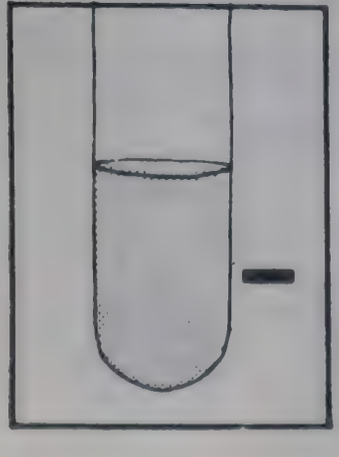


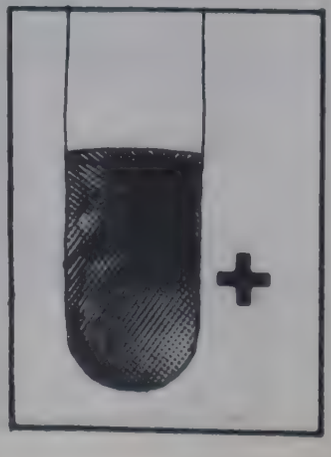
(3) Shake the tube.
Observe the colour produced at once.



Results

Negative result: Faint yellowish-brown colour.
Positive result : Green colour
– pale green : +
– intense green: ++





B. Test using Fouchet reagent

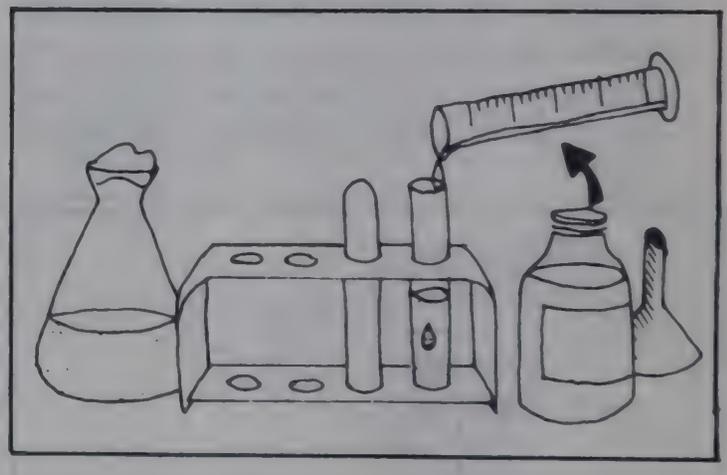
This is a more sensitive test and will confirm results obtained by the iodine test.

Materials

– Test tubes
– Funnel
– Filter paper
– Dropping pipette or drop bottle
– 10 ml measuring cylinder
– 100 g/l (10%) barium chloride aqueous solution (Reagent No. 3)
– Fouchet reagent (Reagent No. 13)

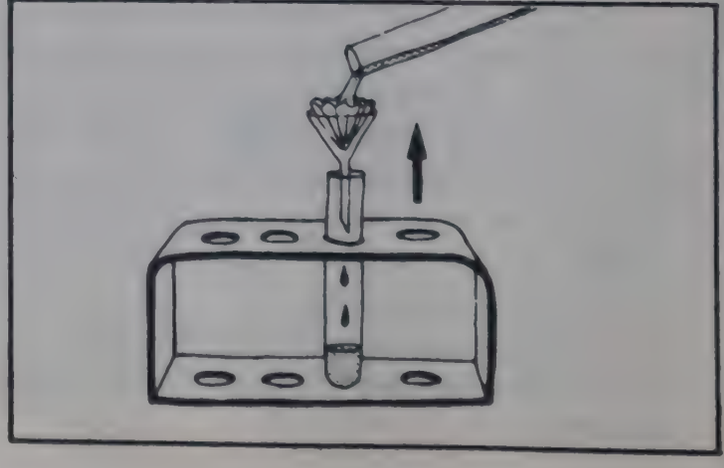
Method

(1) Mix in a test tube:
– 5 ml of urine
– 2.5 ml of barium chloride solution.



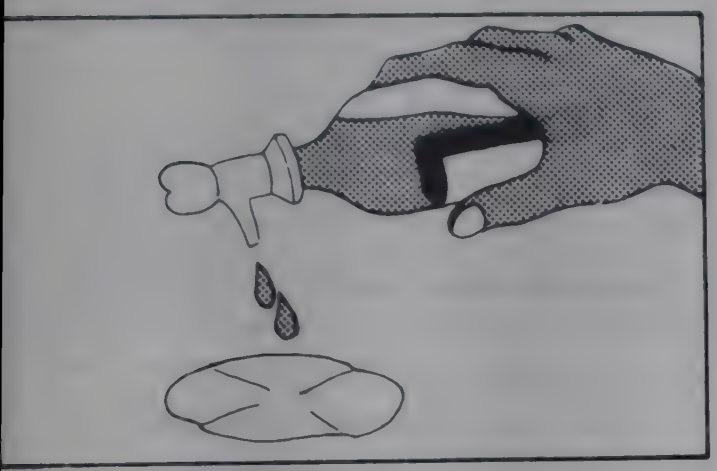
A precipitate forms.

(2) Filter the mixture.



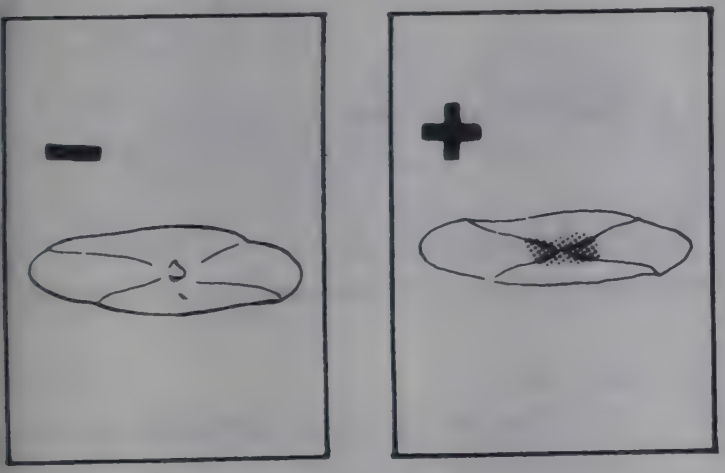
The precipitate which contains the bile pigment remains on the filter paper.

3) Unfold the filter paper.
Add to the precipitate on the filter paper:
– 2 drops of Fouchet reagent.



Results

Negative result: No colour change.
Positive result : The precipitate turns green.



9. Detection of Bile Salts in Urine

Normally bile salts are not found in urine. In certain conditions, e.g. hepatitis, bile salts pass into the blood stream and are excreted in the urine.

Hay's Sulphur powder test

Principle

Bile salts reduce the surface tension of fluids in which they are contained.

Flowers of sulphur normally float on the surface of urine. In the presence of bile salts they will sink.

Materials

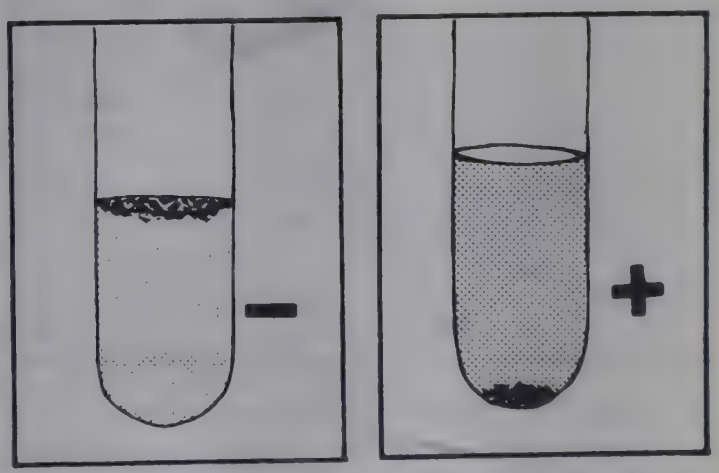
– Test tube and rack
– Sulphur

Method

(1) Cool the urine by placing it in a cool place.
(2) Pour some urine into a test tube.
(3) Sprinkle finely powdered sulphur on the surface of the urine in the test tube.

Results

Bile salts absent (negative result): Sulphur flowers float on the surface.
Bile salts present (positive result): Sulphur flowers sink to the bottom.



10. Detection of Ketone Bodies in Urine

Normal urine does not contain ketone bodies. Acetone and other ketone bodies may appear in the urine:

– in severe or untreated diabetes
– in certain other conditions, e.g. dehydration, vomiting, malnutrition, or after violent exercise.

Ring test

Principle

When sodium nitroprusside is added to urine

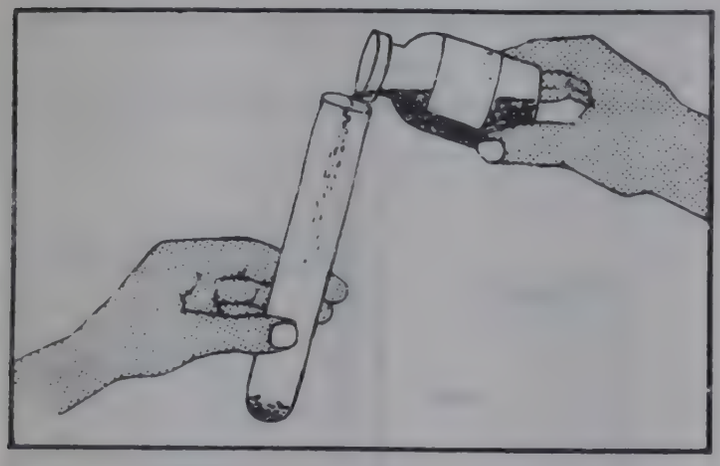
containing ketone bodies, a purple colour is produced.

Materials

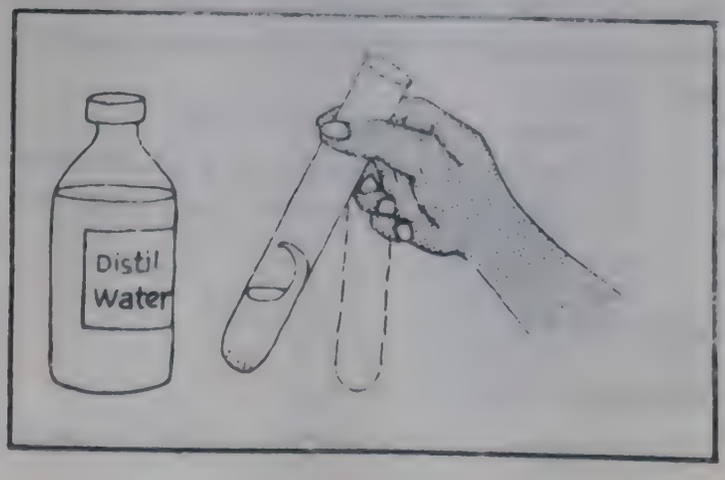
- Test tubes and rack
- 10 ml measuring cylinder
- Dropping pipette
- Sodium nitroprusside crystals
- Glacial acetic acid
- Ammonia

Method

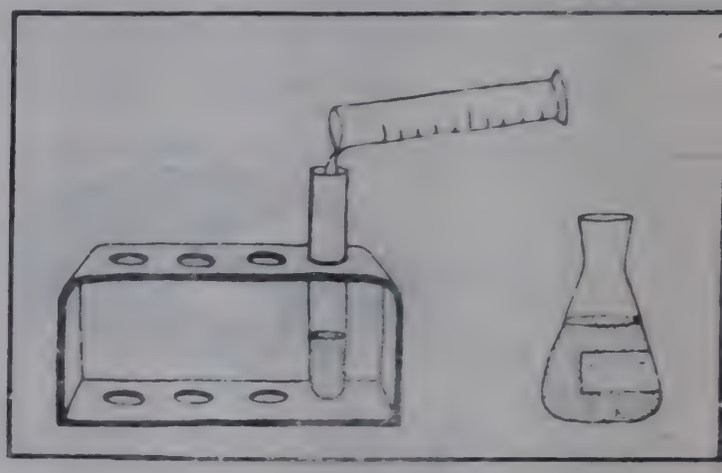
(1) Preparation of sodium nitroprusside solution: Just before carrying out the test, place a few crystals of sodium nitroprusside in a test tube (enough to cover the bottom of the tube).



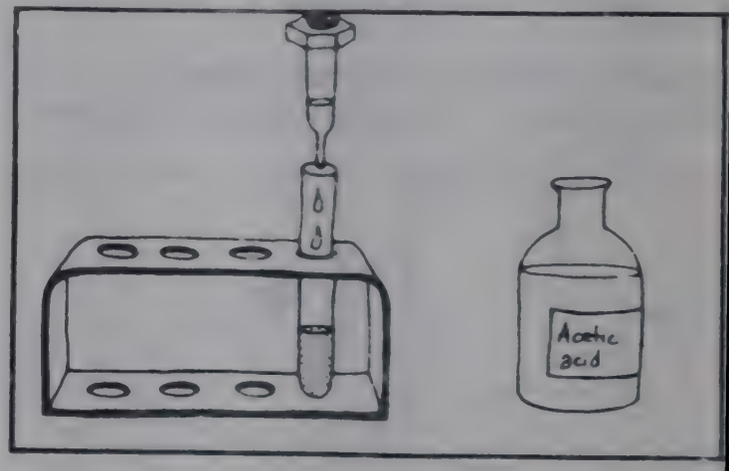
(2) Add 5 ml of distilled water.
Shake well until the crystals are almost dissolved. Not all of the crystals are expected to dissolve as the solution is saturated.



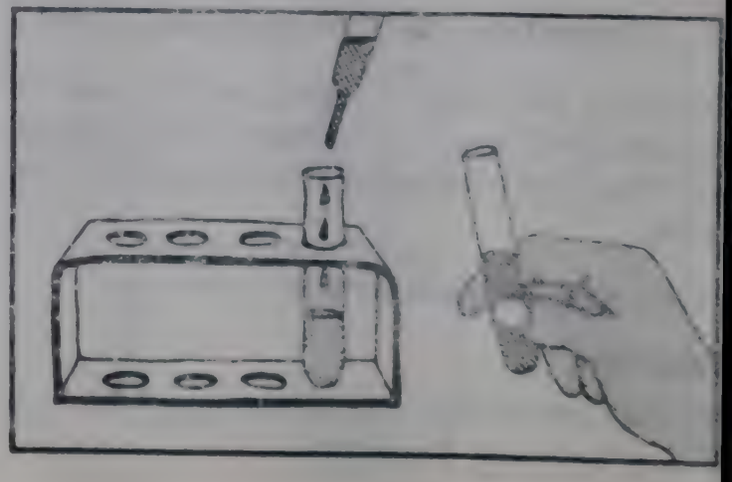
(3) Into another test tube measure:
– 10 ml of urine.



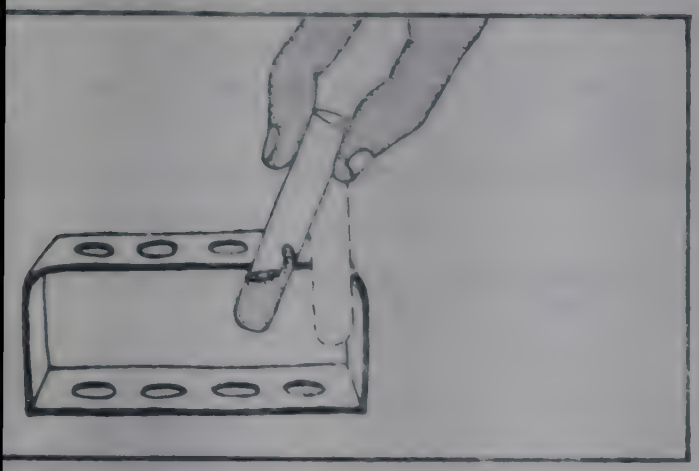
(4) Add to the urine:
– 10 drops of glacial acetic acid.



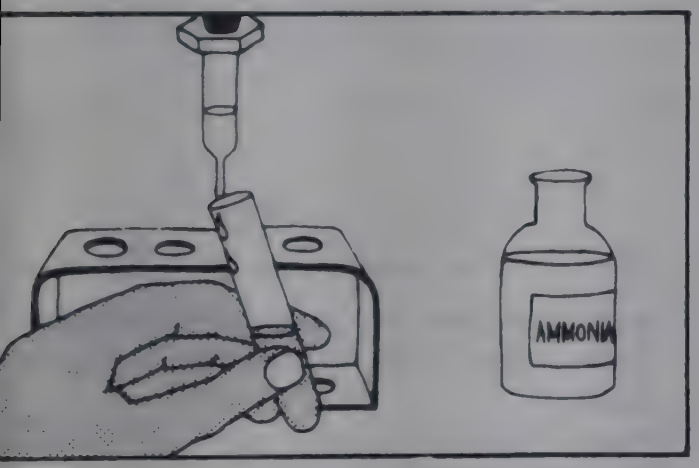
(5) Then add:
– 10 drops of the freshly prepared sodium nitroprusside solution.



) Mix well.



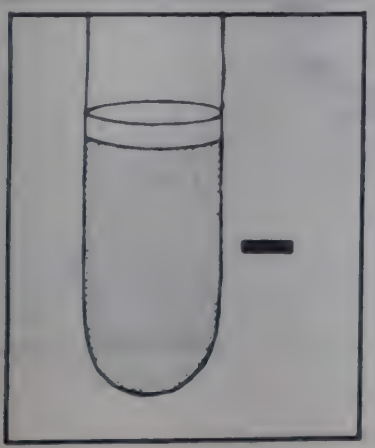
) Holding the tip of the pipette against the side of the tube, let 20 drops (1 ml) of ammonia solution flow on to the surface of the liquid.



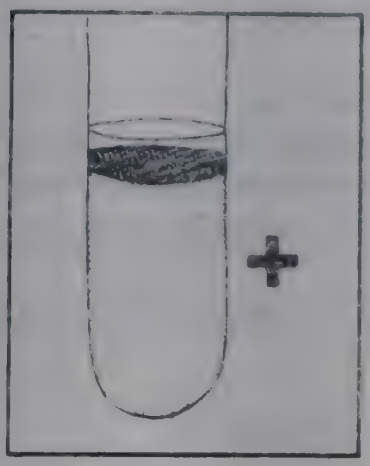
Wait 5 minutes.

esults

Negative result: No change in colour.



Positive result : Purple ring at the top of the urine.
Pinkish ring : +
Red ring : ++
Deep violet ring: +++



11. Microscopic Examination of Urine

A. Urinary deposits

Urine contains microscopic elements in suspension (cells, crystals, etc.). These elements are collected by centrifuging and a drop of the deposit is examined between the slide and coverslip.

As all these elements in suspension would sediment in the urine if left for a few hours, they are called urinary deposits.

Collection of urine

Examine a specimen passed in a single urination.

Examine a mid-stream specimen of fresh urine as soon as possible. It should be:

- collected in the laboratory; *or*
- brought quickly from the patient's home (within 2 hours of voiding).

The receptacle should be provided by the laboratory

Women should be instructed to wash the genitalia beforehand.

Never carry out the examination on urine kept in the refrigerator.

In certain diseases of the urinary tract the urinary deposits are considerably altered. The following elements may be found:

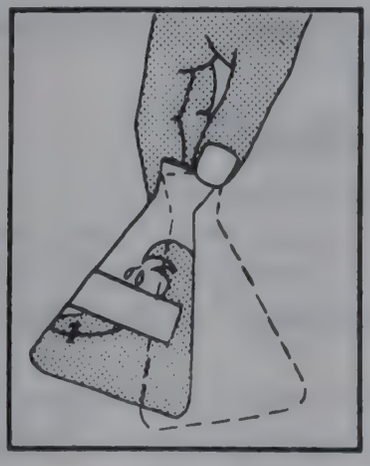
- pus
- an abnormal number of red blood cells
- abnormal crystals
- parasitic forms.

Materials

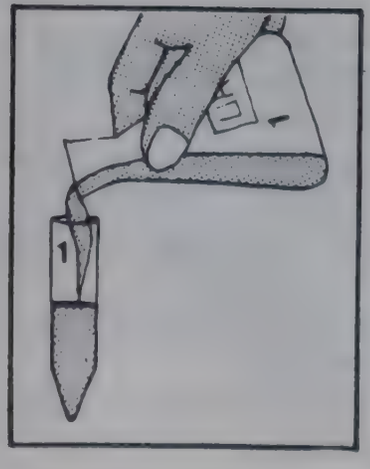
- Hand centrifuge
- 15 ml conical centrifuge tubes
- Capillary dropping pipette (Pasteur pipette)
- if possible calibrated to deliver 50 drops per ml.
- Slide and coversip, 20 × 20 mm

Method

(1) Mix the urine gently.

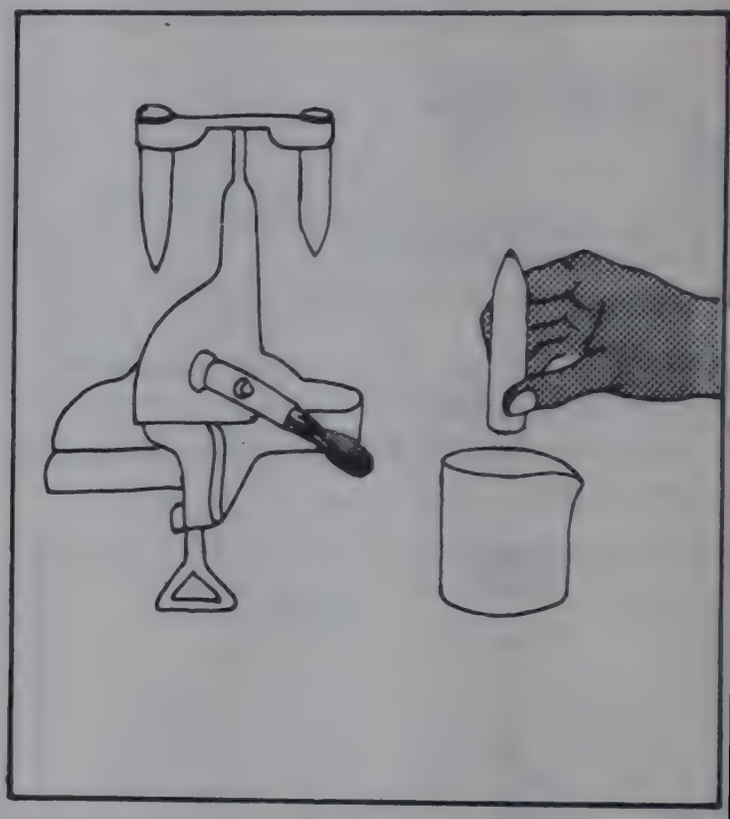


(2) Pour immediately into a centrifuge tube until it is ¾ full.

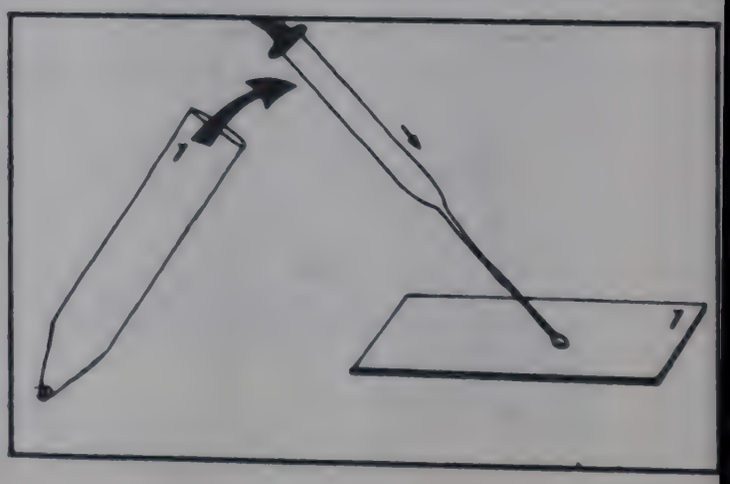


(3) Centrifuge at medium speed for 5 minutes.

(4) Pour off the supernatant urine by inverting the tube quickly without shaking. (The supernatant urine can be used for chemical tests.)



(5) Shake the tube to resuspend the deposit. Draw a few drops of the deposit into a pipette.
Place 1 drop on a slide and cover with a coverslip.
Number the slide with the number of the specimen.



(6) Examine under the microscope at once:
- first using the × 10 objective
- then using the × 40 objective
- with the condenser lowered enough (or the condenser aperture reduced) to make transparent elements visible.

The following may be found in urinary deposits:
- red blood cells
- leucocytes
- yeasts
- trichomonas
- spermatozoa
- epithelial cells
- casts
- parasitic eggs and larvae
- crystals

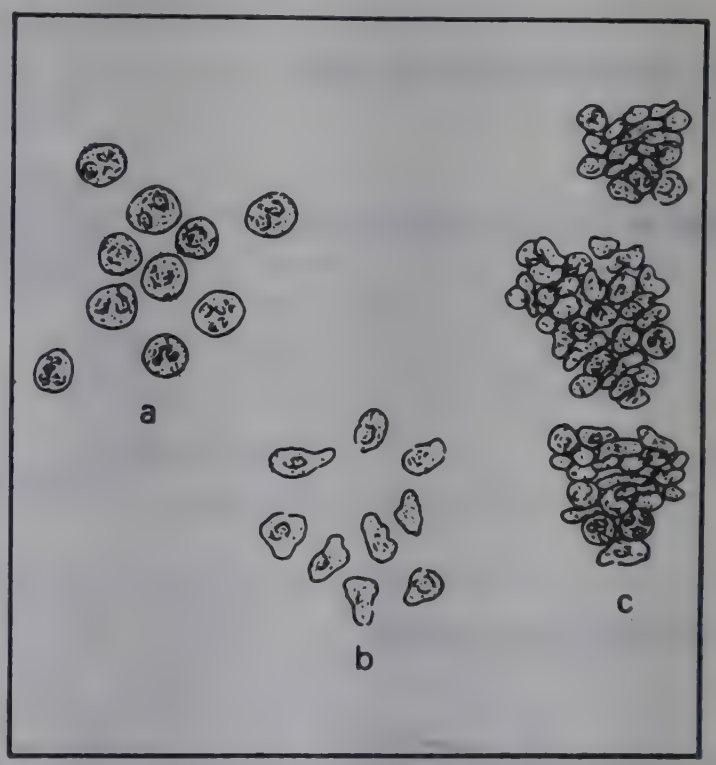
(1) Red blood cells

They may be:
(a) intact: small yellowish discs, darker at the edges (8 μ)
(b) crenated: spiky edges, reduced diameter (5–6 μ)
(c) swollen: thin circles, increased diameter (9–10μ)

There are normally no red blood cells in the urine.

Note: Red blood cells can be found in the urine of women if the specimen has been taken during the menstrual period.

(2) Leucocytes (white blood cells)



They may be:
(a) intact: clear granular discs, 10–15 μ (the nuclei may be visible)
(b) degenerate: distorted shape, shrunken, less granular
((c) pus: clumps of numerous degenerate cells.

The presence of many leucocytes, especially if in clumps, usually indicates a urinary tract infection.

It is important to mention the quantity of the various elements found.

It is important always to use the same method of expressing quantities found.

With: – 1 drop of urine (deposit 1/50 ml)
– 1 coverslip, 20 × 20 mm
– × 40 objective; eyepiece × 5
} examine microscopically:

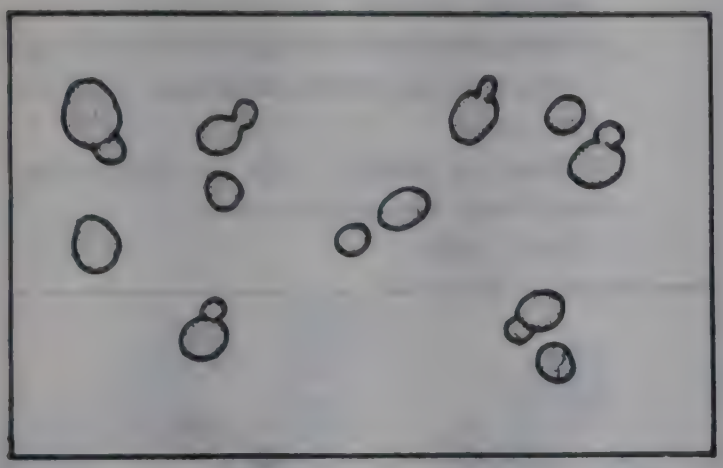
Red blood cells

0–10 red blood cells per field		Few red blood cells (Normal)
10–30 red blood cells per field		Moderate number of red blood cells
Over 30 red blood cells per field		Many red blood cells

Leucocytes

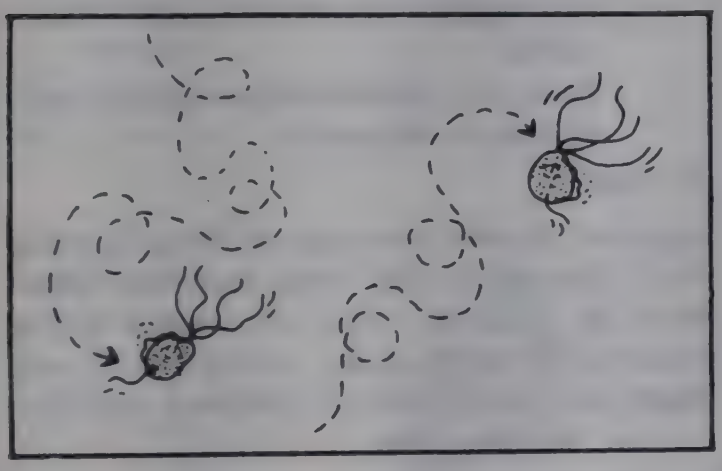
0–10 leucocytes per field		Few leucocytes (Normal)
10–20 leucocytes per field		Moderate number of leucocytes
20–30 leucocytes per field		Many leucocytes
Clumps of more than 20 degenerate leucocytes		Many leucocytes seen in clumps
Clumps and many degenerate leucocytes		Full field

(3) Yeasts



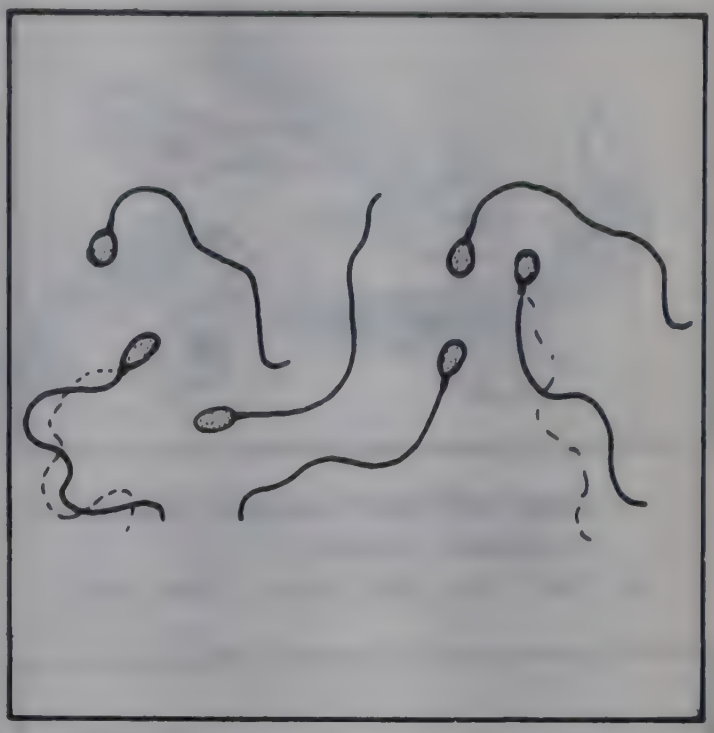
Do not confuse with red blood cells.
Size : 5–12μ
Shape: round or oval bodies of various sizes found together. Budding may be seen.
They are not soluble in acetic acid. Yeasts are occasionally present in urine containing glucose. Check that the urine is fresh.

(4) Trichomonas



Size : 15μ (2 red blood cells)
Shape : round, globular
Motility : motile in fresh urine (they whirl and turn)
Undulating membrane : like the fin of a fish, on one side only, very motile
Flagella : 4 flagella, more or less visible, whiplike, very motile
The whole impression is one of movement.

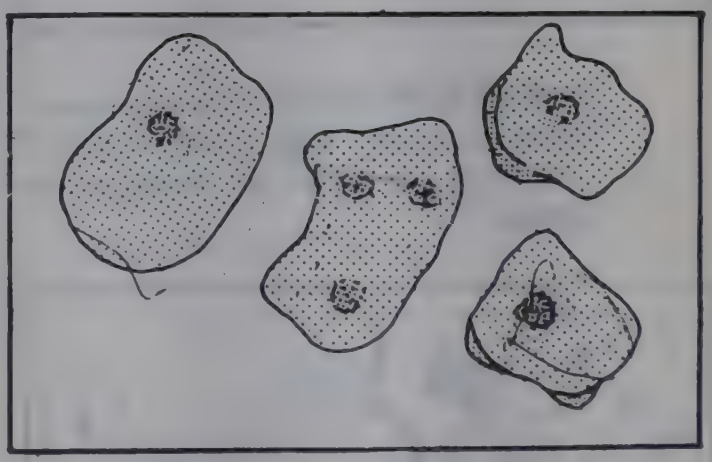
(5) Spermatozoa



Occasionally found in the urine of males.
Head : very small (5 μ)
Flagellum : long and flexible (50 μ)
Motility : motile in very fresh urine.

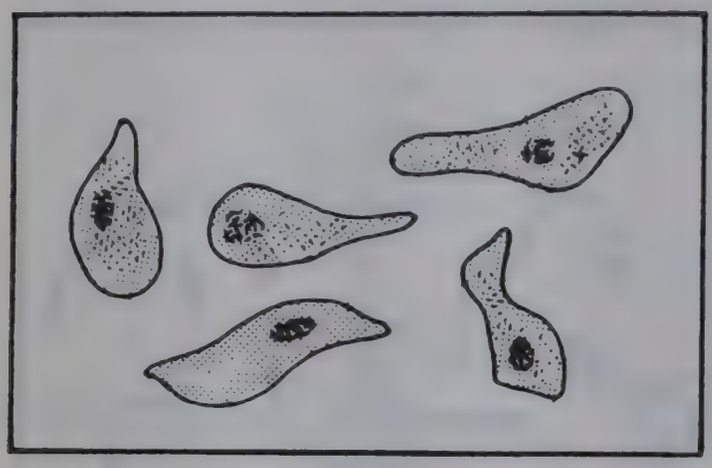
(6) Epithelial cells

i. *Squamous epithelial cells*:



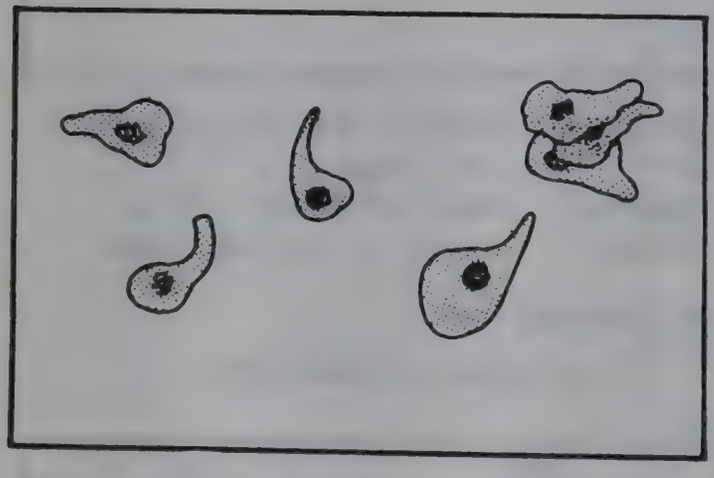
Large rectangular cells, the product of desquamation (the shedding of cells from the epithelium of the urinary tract and organs).
They come from the urethra or the vagina.

ii. *Bladder cells*:



Large cells often diamond-shaped, with a distinct nucleus.

iii. *Cells from the pelvis of the kidney*:



Medium-sized cells (the size of 3 leucocytes), granular, with a sort of tail.

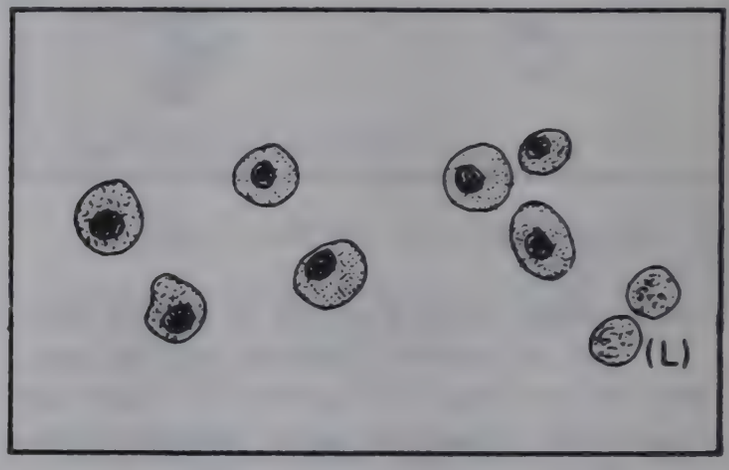
iv. *Cells from the ureter and pelvis of the kidney*:

Medium-sized oval cells with a distinct nucleus.

If many are present together with leucocytes and filaments, they may be from the ureter.

If few are present with no leucocytes, they may be pelvic cells.

v. *Renal cells*:



Renal cells are small. They are:
– the size of 1–2 leucocytes (L)
– very granular.

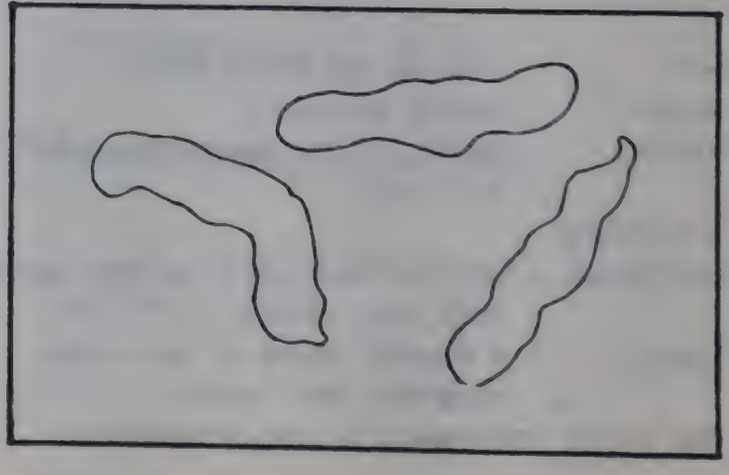
The nucleus is refractile and clearly visible.

They are almost always present with protein in the urine.

(7) Casts

Casts are cylindrical in shape and long, crossing almost the whole field when examined under the × 40 objective. They are formed during disease in the renal tubules, which may fill with blood and other cells and chemical deposits.

i. *Hyaline casts*:



Transparent and slightly refractile, the ends rounded or tapered.
(They may be found in healthy persons after strenuous muscular effort.)

ii. *Granular casts*:

Rather short casts filled with large granules, pale yellow in colour, with rounded ends.
(The granules come from degenerate epithelial cells from the tubules of the kidney.)

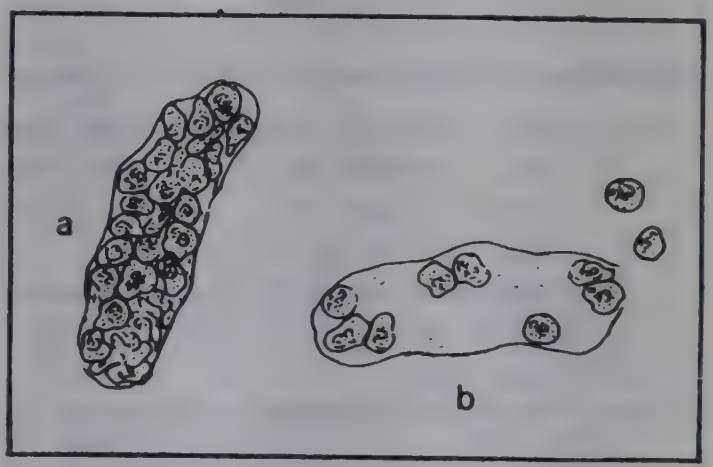
iii. *Fine granular casts*:

The granules are smaller and do not fill the cast.
Do not confuse with hyaline casts (H) partly covered by amorphous phosphate crystals.

iv. *Blood casts*:

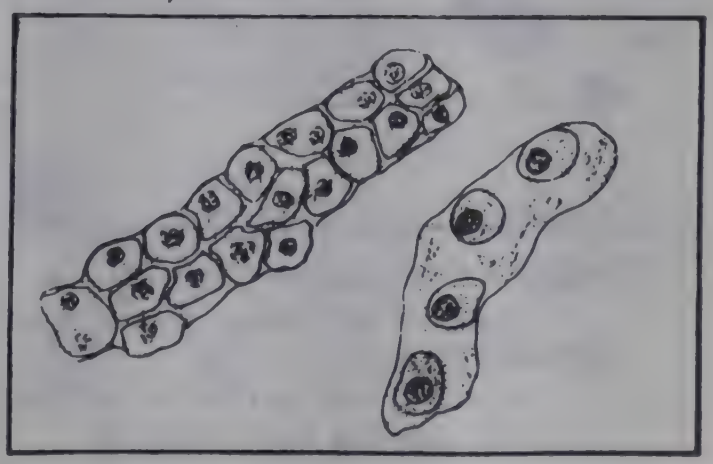
Casts filled with more or less degenerate red blood cells, brownish in colour.

v. *Pus casts*:



Casts filled with degenerate leucocytes. True pus casts are completely filled with leucocytes (a).
Hyaline casts may contain a few leucocytes (b).

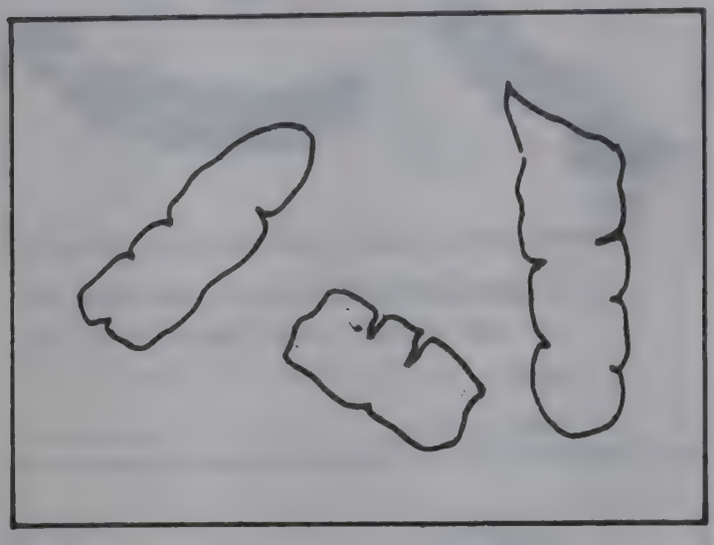
vi. *Epithelial casts*:



Casts filled with pale yellow epithelial cells.

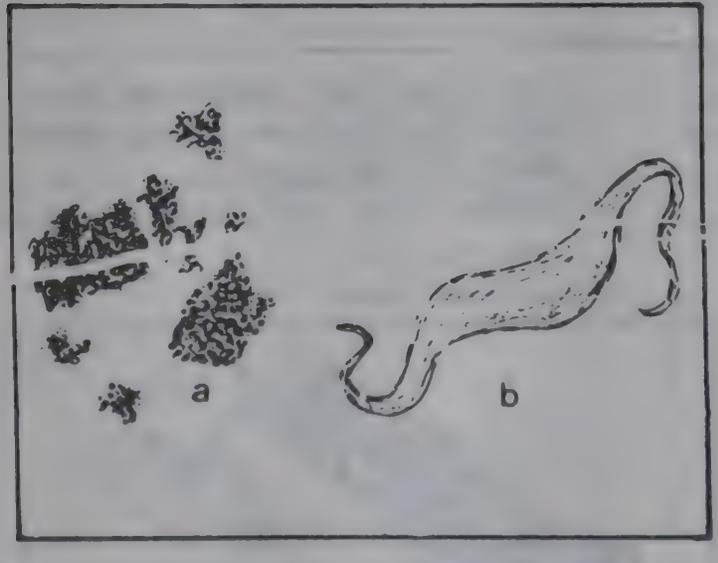
Note: To make the cells more distinct, add a drop of 10% acetic acid to the deposit.

vii. *Fatty casts* (rare):



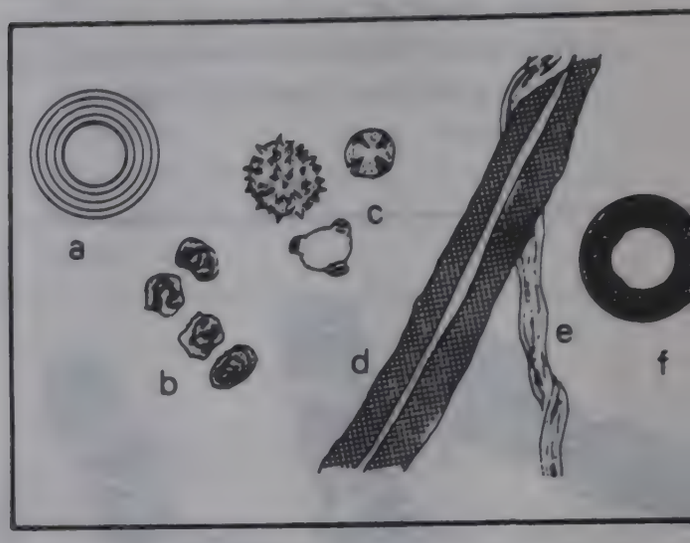
Very refractile yellowish casts, the edges indented and distinct, the ends rounded. Fatty casts are soluble in ether but not in acetic acid.
(They are found in cases of severe kidney disease.)

viii. *False casts*:



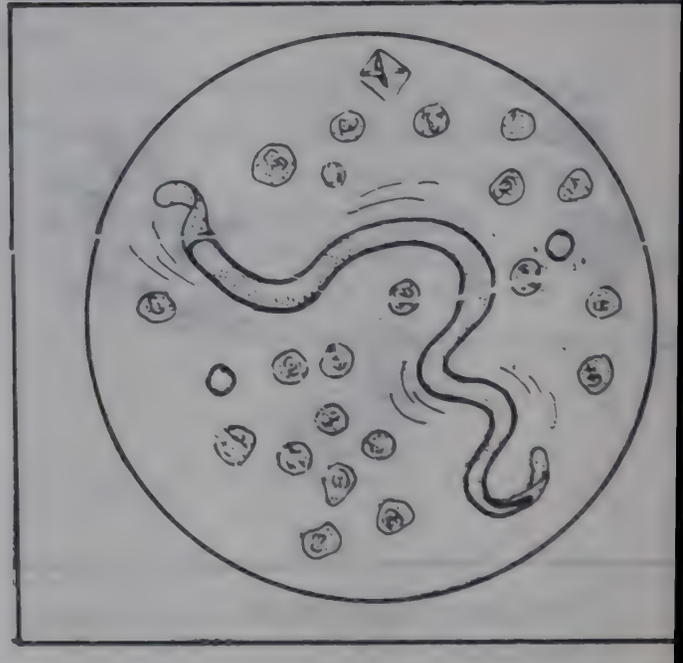
Do not mistake for casts:
- masses of phosphate crystals, short and clear-cut (a)
- masses of translucent mucus, the ends tapering into threads (b).

(8) Miscellaneous foreign substances



If dirty receptacles or slides are used, or if t
urine specimen is left exposed to the air, t
following may be found:
(a) oil droplets (refractile)
(b) starch granules (blue-black with Lug
iodine solution)
(c) grains of pollen from flowers
(d) hairs
(e) cotton fibres
(f) air bubbles.

(9) Microfilariae of Wuchereria bancrofti



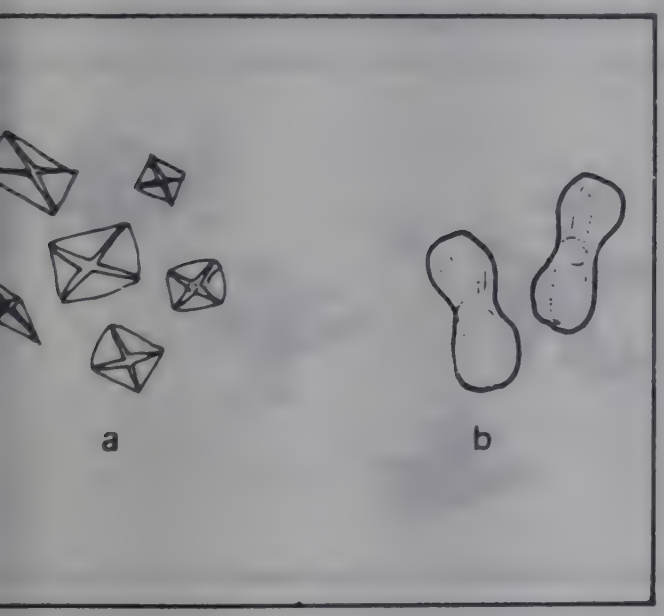
The microfilariae are still motile. They mov
regular curves.
Size: 200–300 μ long, 8μ thick.

sheath of the organism is visible in the
e. Usually large numbers of leucocytes are
present. The urine appears milky because
le is present.

) Crystals

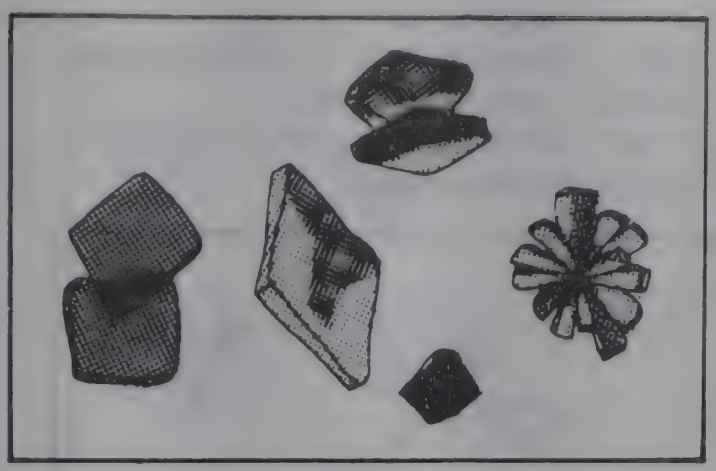
ystals have regular geometric shapes (a),
like amorphous debris, which is made up of
mps of small granules with no definite
pe (b).

Calcium oxalate (acid urine):



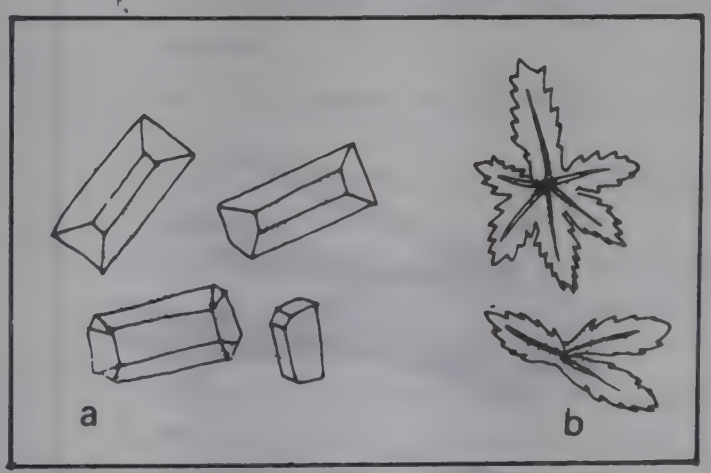
(a) Shape: like an envelope
Size : 10–20 μ (1–2 red blood cells)
or
(b) Shape: like a whole groundnut
Size : about 50 μ, very refractile.

ii. *Uric acid* (acid urine):



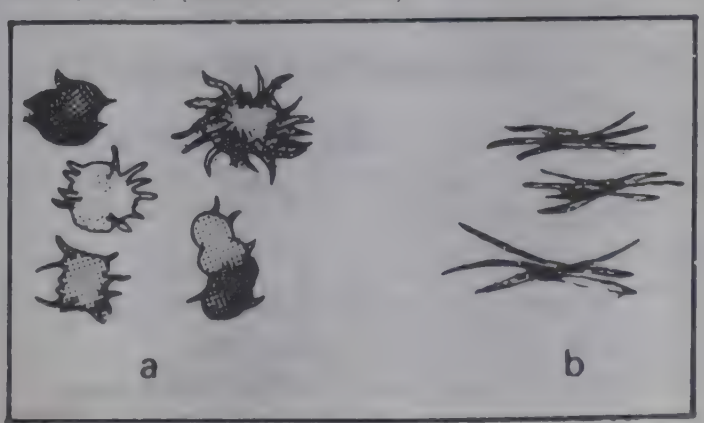
Shape : varies (square, diamond-shaped, cubical or rose-shaped)
Size : 30–150 μ
Colour: yellow or brownish-red.

iii. *Triple phosphates* (neutral or alkaline urine):



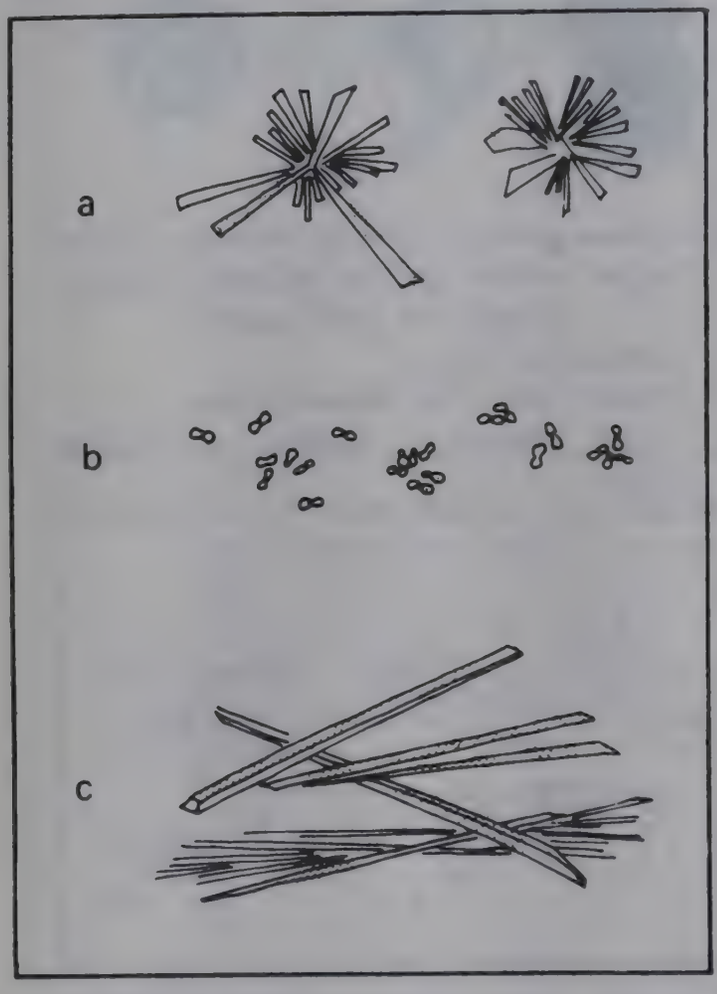
Shape : rectangular (a) or like a fern leaf or star (b)
Size : 30–150 μ
Colour: colourless, refractile.

iv. *Urates* (alkaline urine):



Shape : like a cactus (a) or a bundle of needles (b)
Size : about 20 μ (2–3 red blood cells)
Colour: yellow, refractile.
(Often found together with phosphates.)

v. *Less common crystals:*



(a) *Calcium phosphate* (neutral or alkaline urine)
Shape : star-shaped
Size : 30–40 μ
Colour: none.

(b) *Calcium carbonate* (neutral or alkaline urine):
Crystals: very small like millet grains grouped in pairs
Colour : none.
(If 10% acetic acid is added they dissolve, giving off bubbles of gas.)

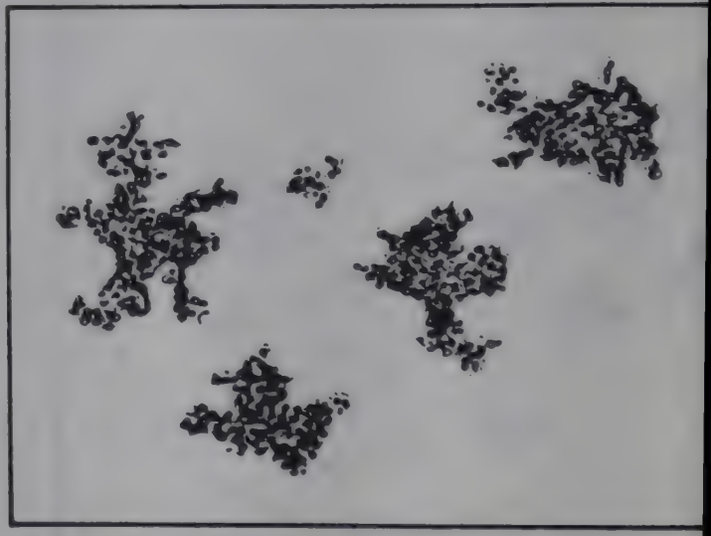
(c) *Calcium sulphate* (acid urine)
Shape: long prisms or flat blades, separate or in long bundles
Size : 50–100 μ
(They can be distinguished from calcium phosphate crystals by measuring the pH of the urine.)

(11) Amorphous debris

i. *Amorphous phosphates* (alkaline urine):

Granules – small, whitish, often scattered.
They are soluble in 10% acetic acid (1 drop per drop of deposit).

ii. *Amorphous urates* (acid urine):



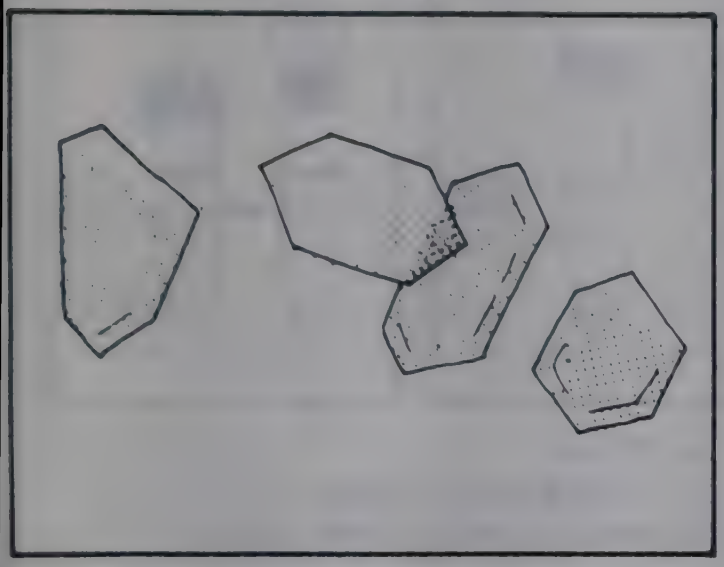
Granules – very small, yellowish, grouped in compact clusters.
They are not soluble in 10% acetic acid, but dissolve if the urine is gently heated.

Note: Urine kept in the refrigerator often shows a heavy precipitate of urates.

(12) Other crystalline deposits

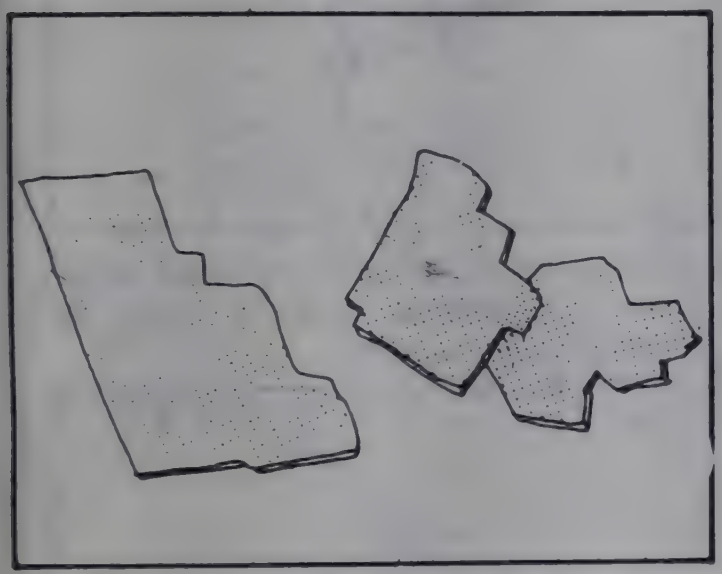
The following are rarely found in the urine. When present, however, they are found in large quantities.

i. *Cystine* (acid urine):



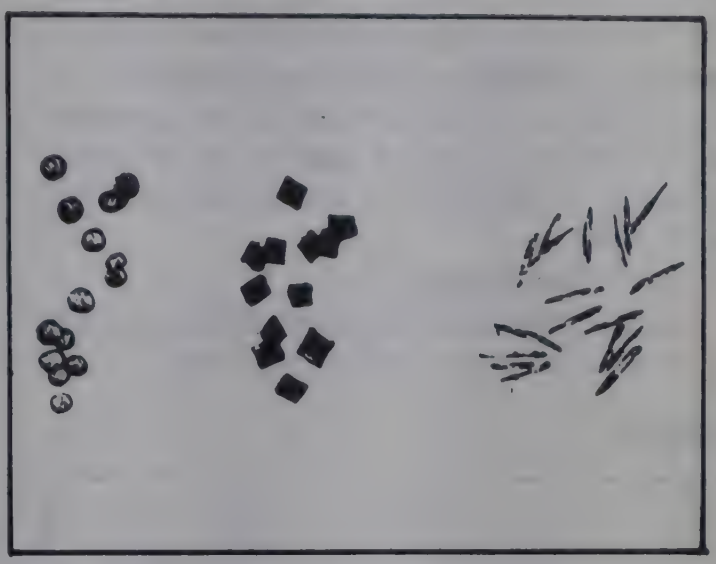
Shape : hexagonal plates
Size : 30 – 60 μ
Colour: colourless, very refractile.
Found only in fresh urine, as they are soluble in ammonia.

ii. *Cholesterol* (acid urine):



Shape : squarish plates, with notches on one side
Size : 50 – 100 μ
Colour: colourless, refractile.
Soluble in ether.

iii. *Bilirubin* (very rare):



Shape : various tiny crystals, square, or like beads or needles
Size : 5 μ (about ½ red blood cell)
Colour: brown.
The chemical test for bile pigments is positive.

iv. *Acetyl sulphonamides* (neutral or acid urine):
Found in patients following treatment with sulphonamide drugs.

Sulphonamide crystals are varied in shape but most frequently like sheaves of needles.

If large quantities of unidentified crystals are seen, find out whether the patient is on sulphonamide therapy.

The presence of these crystals should be reported because they can cause kidney damage.

B. Bacteriological examination

In healthy persons the urine contains practically no organisms. Bacteria may be found:

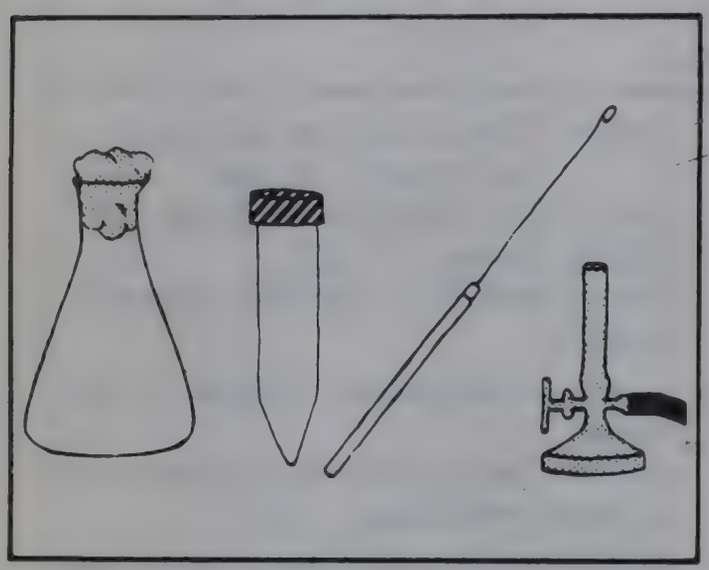
- where there is an infection of some part of the urinary tract (lower tract – urethritis; bladder – cystitis; kidneys – nephritis)
- or where bacteria from an infection elsewhere in the body are excreted in the urine.

Principle

The urine is centrifuged at high speed. Microscopical examination of the urinary sediment as is described under Section A above is essential and the most important part of the analysis. However, the deposit may also be used to make smears that are:

- dried and fixed
- stained by Gram and Ziehl-Neelsen stains
- examined under the microscope.

Materials



- Sterile 250 ml Erlenmeyer flask with stopper
- Centrifuge
- Sterile conical centrifuge tubes with stoppers
- Slides
- Wire loop
- Bunsen burner
- Reagents needed for Gram staining (See Appendix 7.1) and Ziehl-Neelsen staining (See Appendix 10.1).

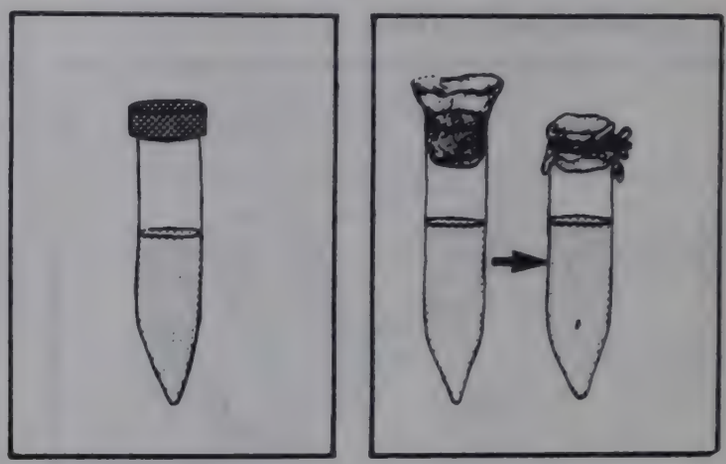
Collection of urine

The genitals should be cleansed beforehand. Collect a mid-stream sample in a sterile flask. Examine as quickly as possible.

Another way is to collect the urine in a conical tube which has been rinsed in boiling water and examine the specimen immediately.

Method

(1) In a sterile tube, stoppered either with a screw cap or with a plug of sterile cotton wool fixed with gauze and string,

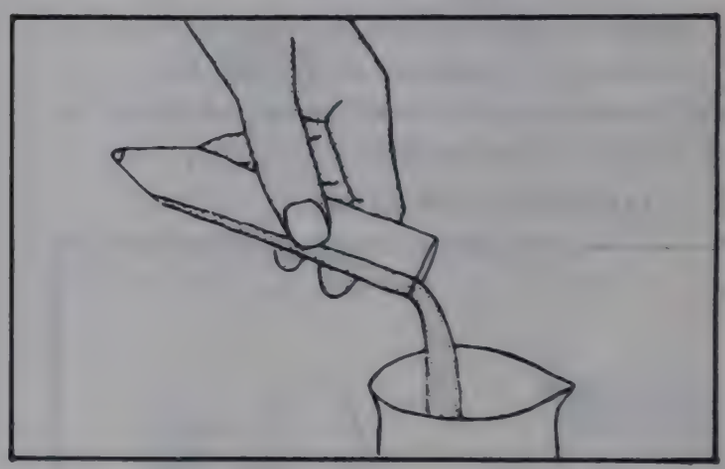


centri fuge:

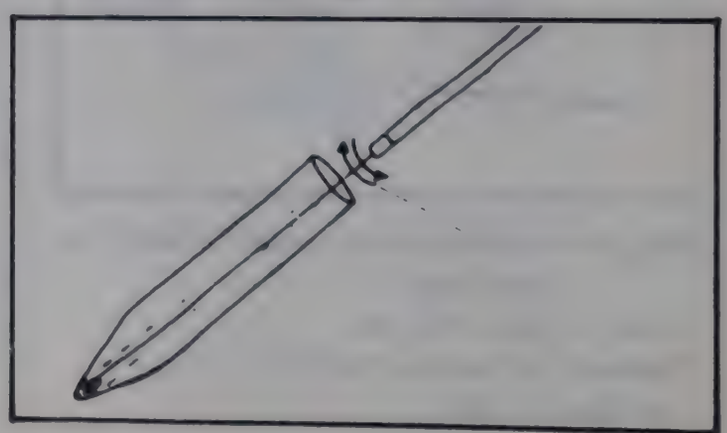
- 10 ml of fresh urine

at average speed for 10 minutes.

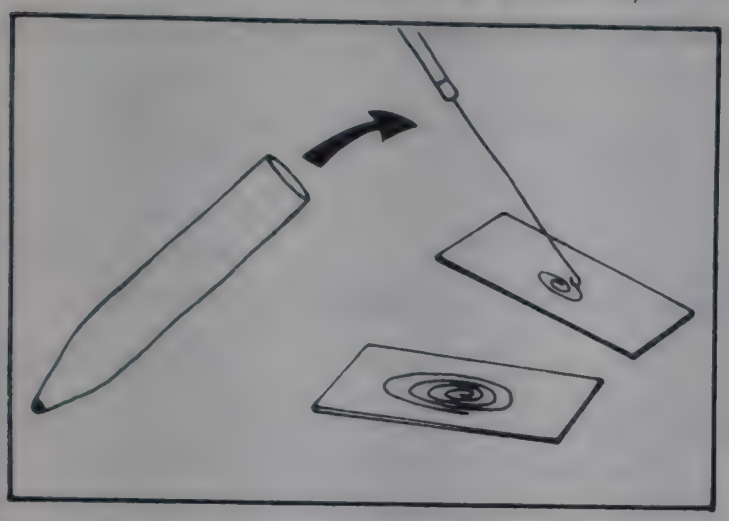
(2) Pour off the supernatant urine.



(3) Mix the deposit, using the wire loop (sterilized by flaming), until it forms a homogeneous suspension.



(4) Make 2 smears. Let the 2 slides dry.

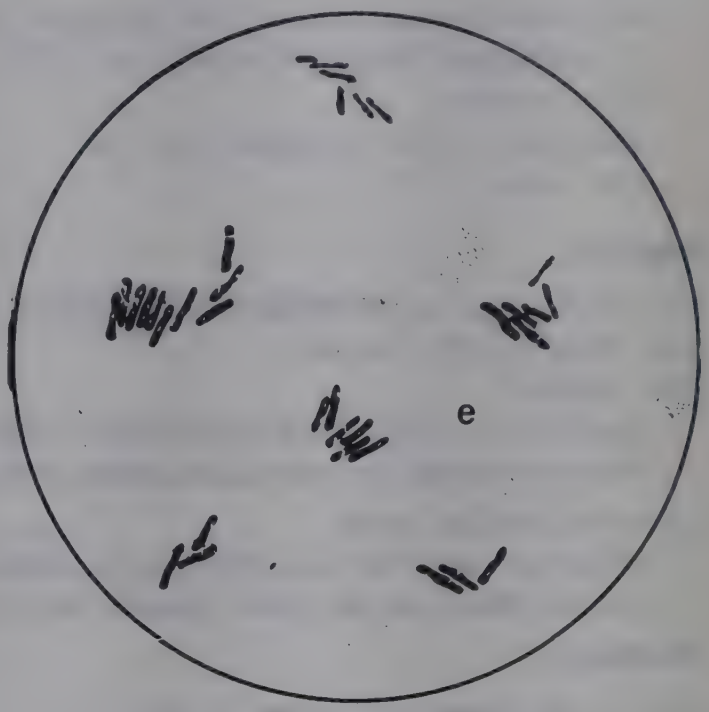


Fix by flooding with ethanol and flaming or by heating

(5) Stain by:
slide 1: Gram stain (See Appendix 7.1)
slide 2: Ziehl-Neelsen stain (See Appendix 10.1)

(6) Examine under the microscope (× 100 objective).
Look for pus: many leucocytes stained red by Gram stain.
Look for organisms such as:

(a) Gram negative bacilli
(b) Gram positive cocci
(c) Gram positive diphtheroid bacilli
(d) Gram positive yeasts



(e) Tubercle bacilli

Note: If the urine is to be examined for tubercle bacilli, centrifuge 10 ml of urine at high speed for 20 minutes

The bacilli stain red with Ziehl-Neelsen stain. They are arranged in rows.

Results

State whether there are leucocytes or pus present.

Give a precise description of the organisms found.

For example, a report may read thus:

Many leucocytes
A few red blood cells
A few epithelial cells
Many Gram positive cocci in clusters.

Appendix 7.1: Gram Staining

Gram staining makes it possible to classify bacteria into two groups:
- Gram positive: stained dark purple
- Gram negative: stained pink.

This makes identification easier.

Reagents

- Modified Hucker crystal violet (Reagent No. 9)
- Gram iodine solution (Reagent No. 15)
- 95% ethanol
- Safranine solution (Reagent No. 24)
- Tap water

Principle

The violet colour stains all bacteria deep violet. Iodine solution fixes the violet colour more or less strongly in the bacteria.

95% ethanol
- decolorizes certain bacteria when the violet stain is not strongly fixed by iodine solution;
- does not decolorize other bacteria when the violet stain is strongly fixed by iodine solution.

Safranine solution (pink)
- re-stains (pink) the bacteria discoloured by ethanol;
- has no effect on the other bacteria which remain dark violet.

Technique

(1) Fix the smear and allow to cool.

(2) Crystal violet – 1 minute:
Pour the crystal violet on to the slide. Cover the slide completely. Leave for 1 minute.
Rinse with tap water and drain.

(3) Gram iodine solution – 1 minute:
Flood the slide with Gram iodine solution and let it stand for 1 minute.
Drain off the solution and rinse with tap water.

(4) 95% ethanol – 1 minute:
Cover the slide completely.
Leave for 1 minute.
Flood with water and drain.
Look at the smear: if violet patches remain, treat again with ethanol for 15 to 30 seconds.
Rinse well with water and drain.

(5) Safranine solution – 10 seconds:
Leave on the slide for 10 seconds.
Wash briefly with tap water once.
Drain and allow to dry in the air.

What to look for

Bacteria stained deep violet:
Gram positive
e.g. staphylococci, streptococci, micrococci, pneumococci, enterococci, diphtheria bacilli, anthrax bacilli

Bacteria stained pink:
Gram negative
e.g. gonococci, meningococci, coliform bacilli, shigellae, salmonellae, cholera vibrios

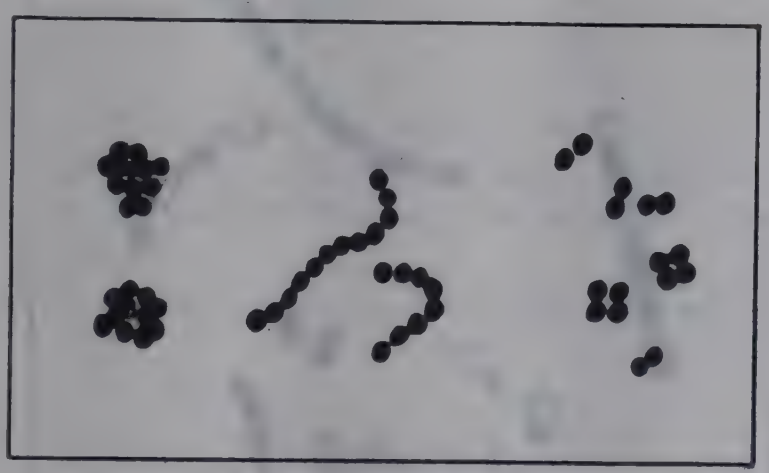
Sources of error

A false Gram positive reaction may occur because:
– The smear was fixed before it was dry.
– The smear was too thick.
– There was sediment in the bottle of crystal violet (filter before using).
– The Gram iodine solution was not thoroughly drained off.
– The ethanol was not left long enough.
– The safranine solution was too strong or was left on the slide too long.

A false Gram negative reaction may occur because:
– The Gram iodine solution was not left long enough.
– The ethanol was left on too long and not washed off properly.

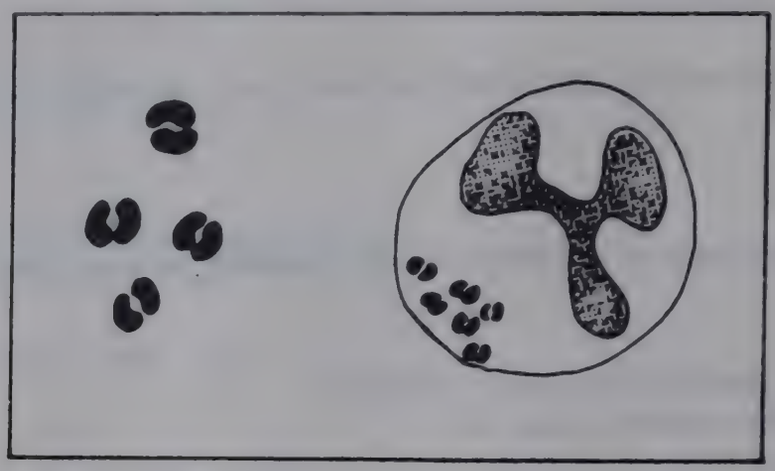
Bacteria seen by Gram stain

1) Gram positive cocci – rounded shape



May be arranged:
– in clusters (staphylococci)
– in chains (streptococci)
– in pairs
– in fours, etc.
Found in pus, urine, blood and other specimens.

(2) Gram negative diplococci – rounded shape in pairs



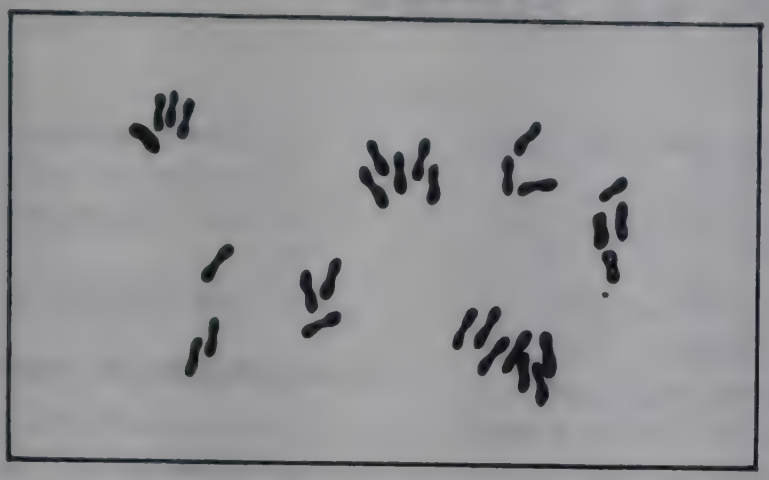
May:
– be shaped like coffee beans
– cluster in the cytoplasm of the leucocyte
Found in urethral pus (gonococci) and the CSF (meningococci).

There are other Gram negative diplococci that are generally non-pathogenic. They may be seen in throat swabs or sputum specimens.

(3) Gram positive bacilli – rod shaped with spores

Long and thick and may have square ends (anthrax) or rounded ends (tetanus). The spore appears as a large uncoloured area inside the bacillus as it does not stain with Gram stain.

Gram positive bacilli – rod shaped without spores



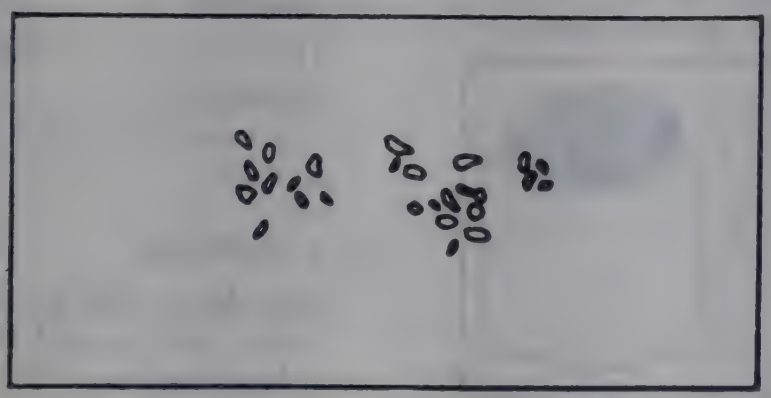
Usually small and variable in shape. The ends may be swollen. The bacilli may be arranged in rows or like letters.

Found in throat specimens, blood, skin, e.g. diphtheria bacilli.

Gram negative bacilli

Variable in size, ends rounded or pointed. May be large and straight (coliform bacilli), comma-shaped (vibrio) or short and fat (Proteus). This group includes many species.

Gram negative coccobacilli



Quite variable in shape, not as round as cocci but not as long as normal bacilli. Found in a variety of body specimens.

CHAPTER 8

Examination of Stools

The following stool examinations are carried out in the PHC laboratory:

- The appearance of the stool (macroscopic examination)
- Microscopic examination for parasites, i.e.
  (a) eggs or larvae of worms
  (b) protozoa, which may be found in a motile (vegetative) form, or in a non-motile, resistant form as cysts.

1. Collection of Stools

The reliability of the results obtained will depend largely on the care taken in collecting the stools. The following precautions should be taken in collecting stools for parasitological examination.

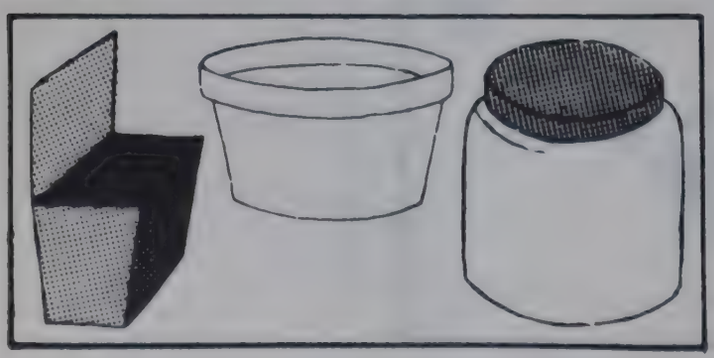
(1) Collection of a sufficient quantity

This is necessary in order:
- to permit detection of parasites, if in low concentration
- to prevent rapid drying of stools.

The specimen should contain at least 4 ml.

(2) Provision of a container for the patient's use

Every effort should be made to give the patient one of the following types of container for collection of the specimen:



- a waxed cardboard box
- an empty tin with a lid
- a clean wide-mouthed glass bottle with a lid
- a wide-mouthed plastic box with a lid

(3) Examination of stools while fresh

- Stools must be examined within one hour of collection.
- If a number of specimens are received at the same time, select liquid stools and those containing mucus or blood and examine these first, for they may contain motile amoebae that die quickly.

Things not to do

(1) Never leave stool specimens exposed to the air in containers without lids.
(2) Never set aside stool specimens for examination at the end of the morning, i.e. 2 or 3 hours later.
(3) Never accept stools mixed with urine, e.g. in a chamber pot or bedpan.
(4) Never place the container with the stool specimen on the examination request form.

2. Appearance of Stools

(1) Quantity

This may be recorded as 'sufficient' or 'insufficient'.
The minimum quantity required for testing is 4 ml.

(2) Consistency

The stools may be:
- firm and formed (normal)
- hard and dry

– soft and formed
– soft and unformed
– semi-liquid (muddy)
– liquid and watery.

(3) Colour

This may be:
– light to dark brown
– black or tarry (indicating bleeding in the upper gastro-intestinal tract)

Note: the stool can also appear black if the patient is taking iron.

– clay-coloured or pale (indicating lack of bile in the intestinal canal)
– white (the stool is temporarily white in colour after a barium meal).

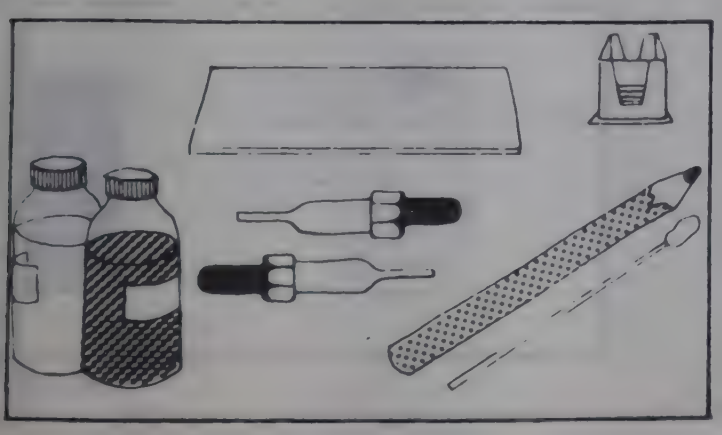
(4) Abnormal features

The following may be seen with the naked eye:
– flakes of mucus (colourless slimy substance like phlegm)
– mucous membranes
– bloodstained mucus
– streaks of pus
– blood superimposed on the stools, which then are red in parts
– worms or segments of worms.

3. Preparation for Microscopic Examination of Stools

A. Slide preparation

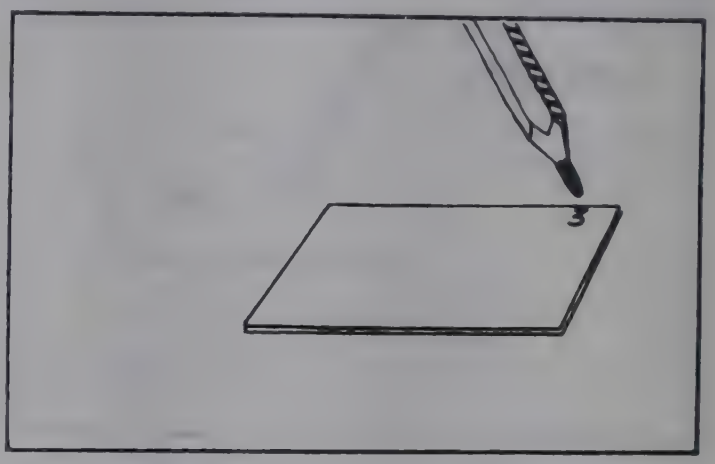
Materials



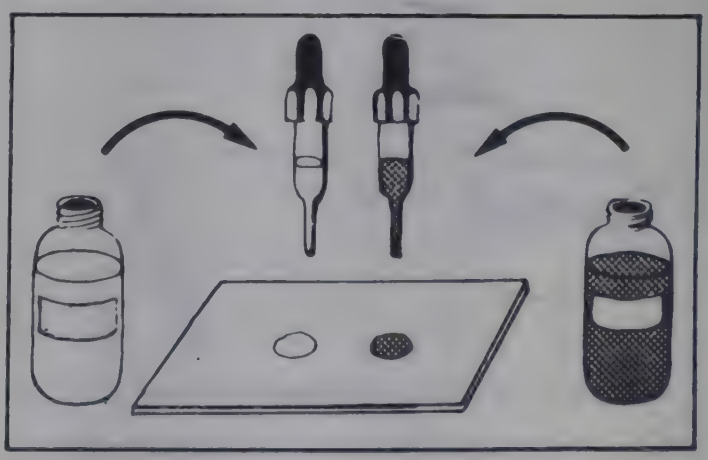
– Microscope slides
– Coverslips 20 mm × 20 mm
– Wooden applicators or wire loops (0.46 mm nickel-chromium alloy wire)
– Grease pencils
– Dropping pipettes
– Sodium chloride solution (Reagent No. 25)
– Lugol iodine solution (Reagent No. 19), diluted 5 times.

Method

(1) Mark the number of the specimen on the slide with a grease pencil.



(2) Place on the slide:
– 1 drop of sodium chloride in the middle of the left half.
– 1 drop of the iodine solution in the middle of the right half.

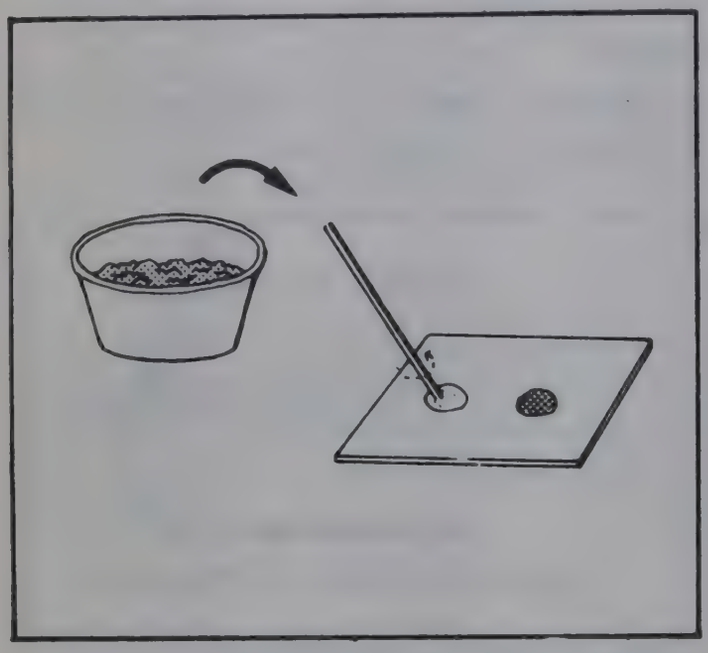


(3) Using an applicator or wire loop, take a small portion (about this size: ●) of the stool. If the stools are formed, take the

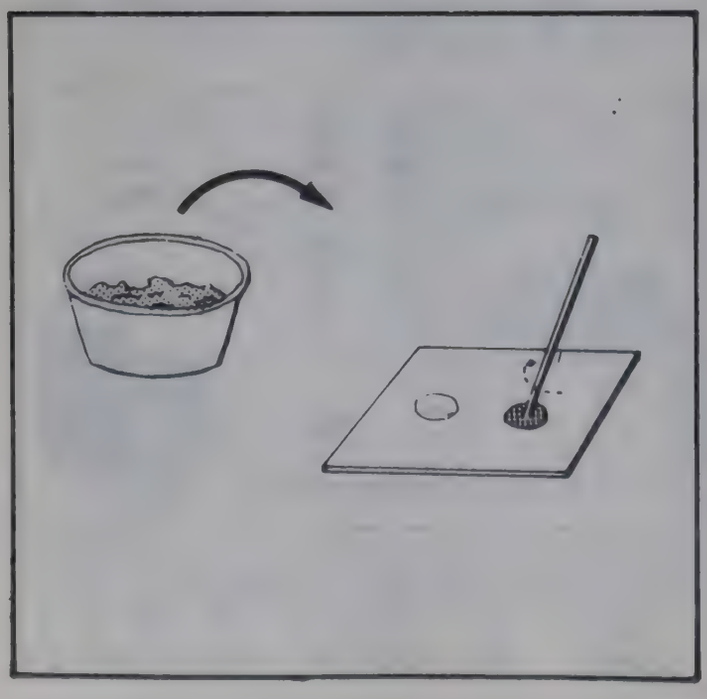
portion from well inside the sample (parasite eggs?) and from the surface.

If the stools contain mucus or are liquid, take the portion from the bloodstained mucus on the surface or from the surface of the liquid (amoebae?).

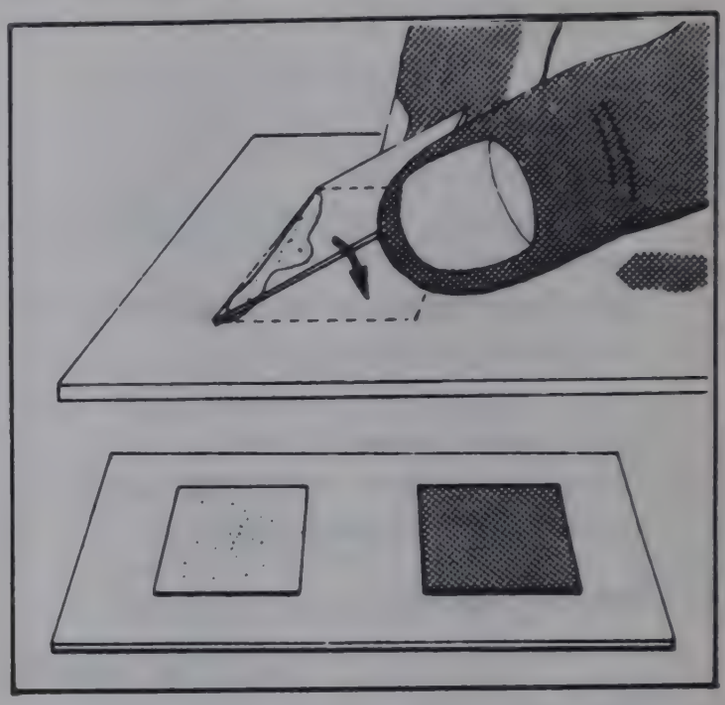
(4) Mix the sample with the drop of sodium chloride solution on the slide.



(5) Using the applicator or wire loop, take a second portion of stool from the specimen and mix it with the drop of the iodine solution.

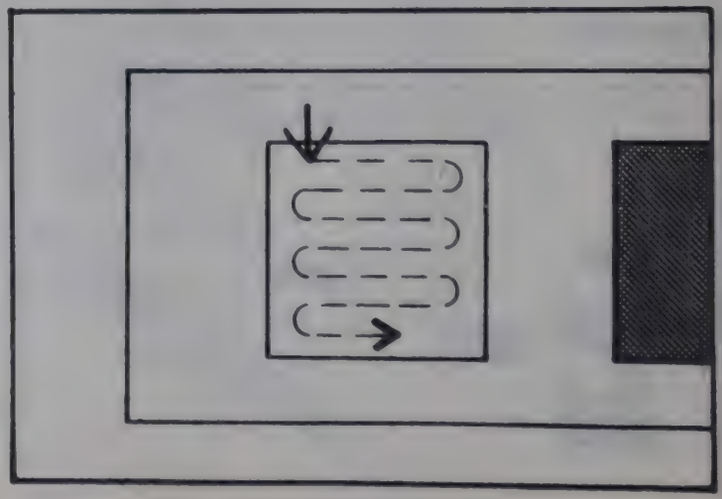


(6) Place a coverslip over each drop. Apply coverslips as shown to avoid the formation of air bubbles.

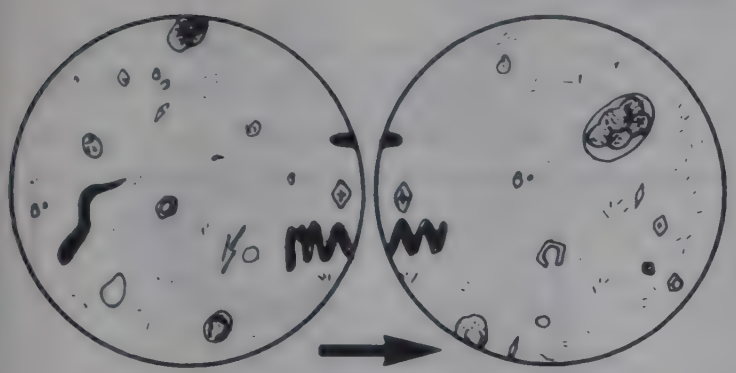


(7) Examine the preparations under the microscope.
For the saline preparation use × 10 and × 40 objectives and the × 5 eyepiece.
For the iodine solution preparation use a × 40 objective.
As the eggs and cysts are colourless, reduce the amount of light using the condenser aperture or lower the condenser to increase the contrast.

(8) Examine the first preparation with the × 10 objective, starting at the top left-hand corner as indicated.



To ensure that no field is overlooked, pick an object on the edge of the field of view and move the slide across the microscope stage, examining the field, until the object reaches the other edge of the field. Repeat the procedure over the whole area.



For each field examined change at least once to the × 40 objective to check for the presence of protozoa, which are very small. Then examine the iodine solution preparation with the × 40 objective.

B. Special technique for pinworm eggs

Principle

The eggs of the pinworm are usually collected (particularly in children) in the folds of skin around the anus. They rarely appear in the stools.

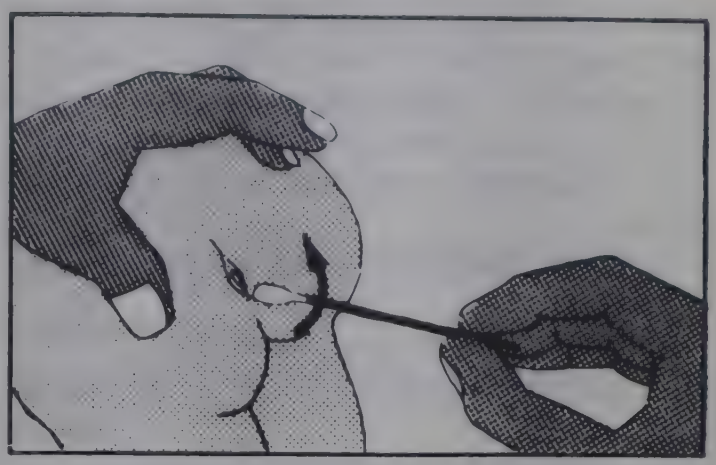
Materials

- Cotton wool swab
- Test tube containing sodium chloride solution (Reagent No. 25)
- Pasteur pipette
- Slide
- Coverslip
- Microscope

Method

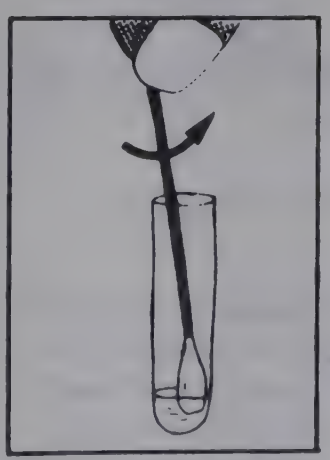
(1) Take a cotton wool swab.

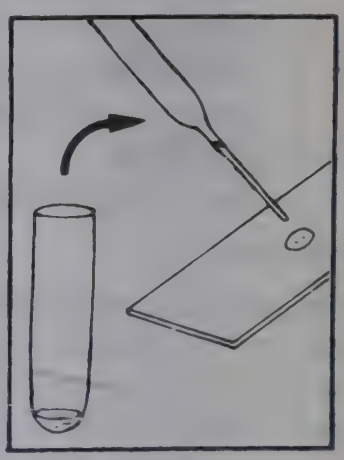
(2) Wipe round the anus (but not inside) with the swab.



(3) Dip the swab into a test tube containing about 0.5 ml (10 drops) of sodium chloride solution. Rinse the swab well in the solution.

(4) Draw up the liquid with a Pasteur pipette. Transfer it to a slide. Cover with a coverslip.





(5) Examine with the × 10 objective as described in step (8) of Section A, Slide preparation (p. 96).

C. Method for concentration of parasites

Concentration of parasites, sometimes called the 'enrichment technique', makes it possible to:

- examine a greater quantity of stools in less volume

– detect parasites present in very small numbers.

Note: A direct microscopical examination of stools must always be made before preparing a concentration. Motile forms of protozoa are not found in concentrated preparations.

Two concentration techniques are described below:

(1) Willis sodium chloride solution technique
(2) Formaldehyde-ether technique

The appropriate technique is chosen according to:

– the equipment and reagents available
– the parasites sought
– the time available.

(1) Concentration method using Sodium Chloride Solution (Willis)

Recommended for:

Eggs of hookworm, roundworm, tapeworm, whipworm.

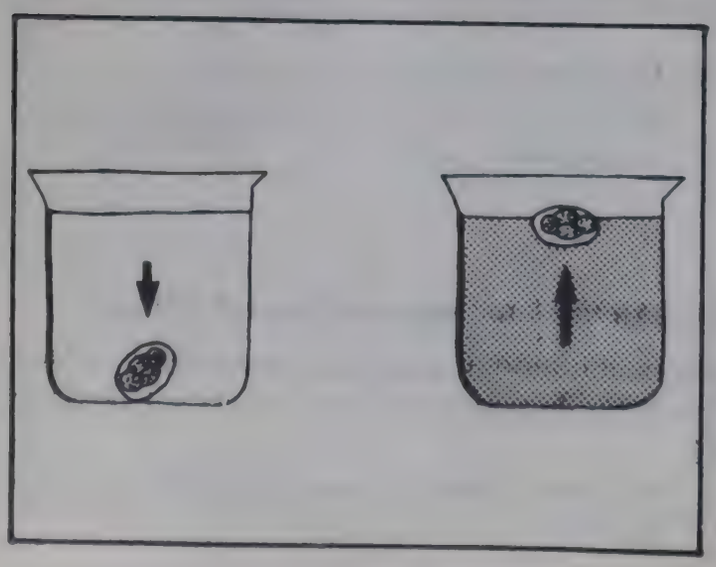
This is the best method for detecting the presence of hookworm.

Not suitable for:

Larvae of Strongyloides, protozoa.

Principle

The stools are mixed with a saturated solution of sodium chloride (increasing the specific gravity). The eggs are lighter in weight and float to the surface, where they can be collected.

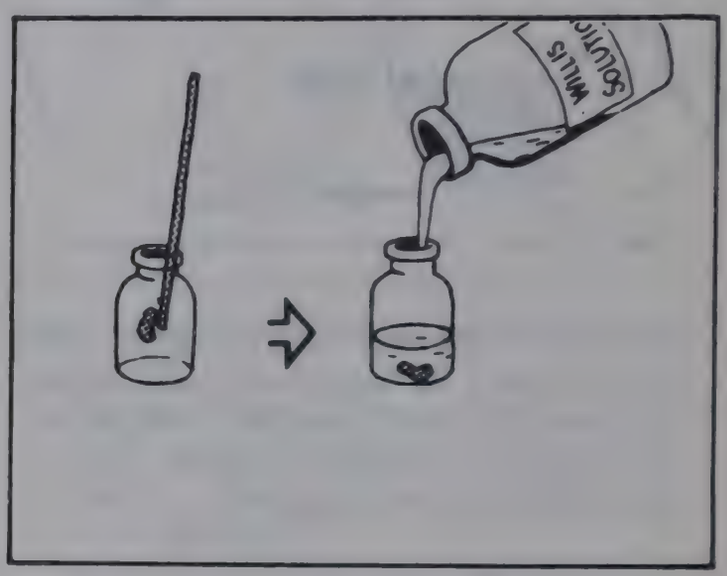


Materials

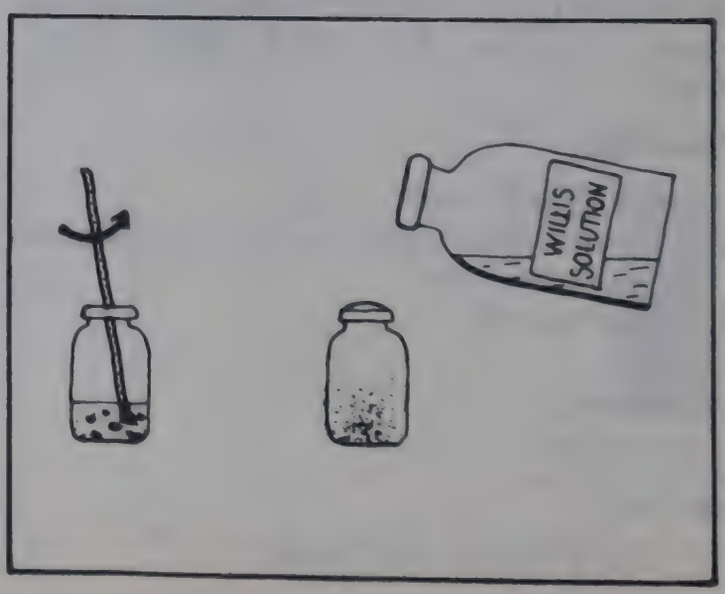
– 10 ml (penicillin) bottles
– Wooden applicators
– Grease-free glass coverslips (See Chapter 1)
– Slides
– Willis solution (Reagent No. 29)
– Petroleum jelly (vaseline) and wax

Method

i. Place a portion of stool (approximately 2 ml) in a penicillin bottle. Quarter-fill the bottle with Willis solution.

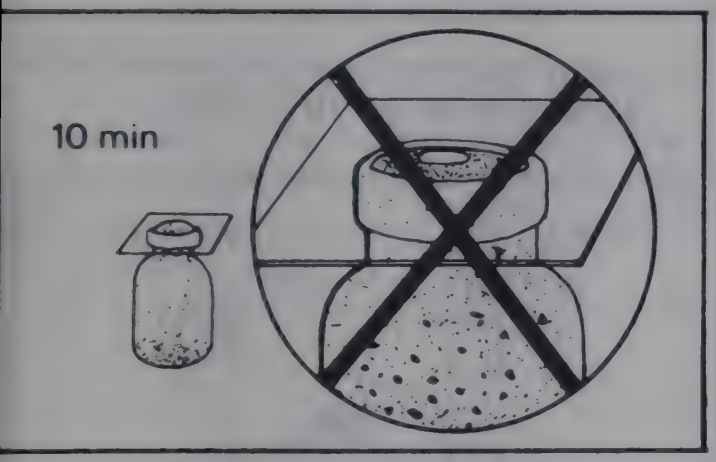


ii. Using an applicator, crush the portion of stool and mix it well with the solution. Then fill the bottle to the top with Willis solution. The suspension should be completely uniform.

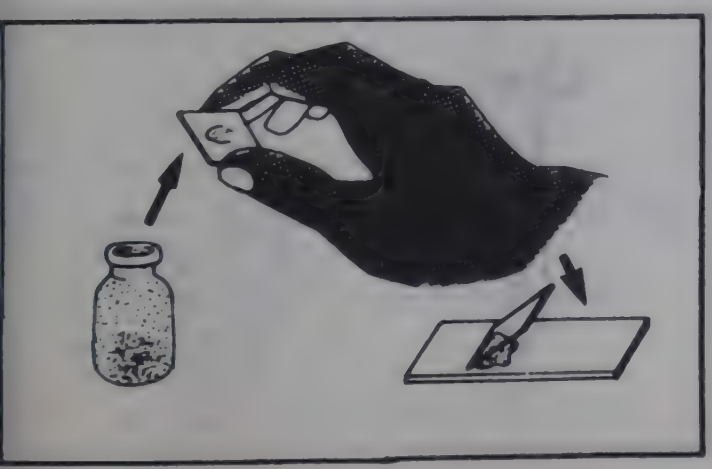


iii. Place a coverslip carefully over the mouth of the bottle.

iv. Check that the coverslip is in contact with the liquid, with no air bubbles. Leave for 10 minutes.



v. Remove the coverslip with care; a drop of liquid should remain on it.
Place the coverslip on a slide and examine under the microscope at once, for the preparation dries very quickly. Otherwise seal the coverslip with petroleum jelly and wax.



vi. Use the fine adjustment of the microscope to examine every object visible in the field (for eggs tend to stick to the coverslip and are not immediately distinct).

(2) Concentration method using Formaldehyde-Ether

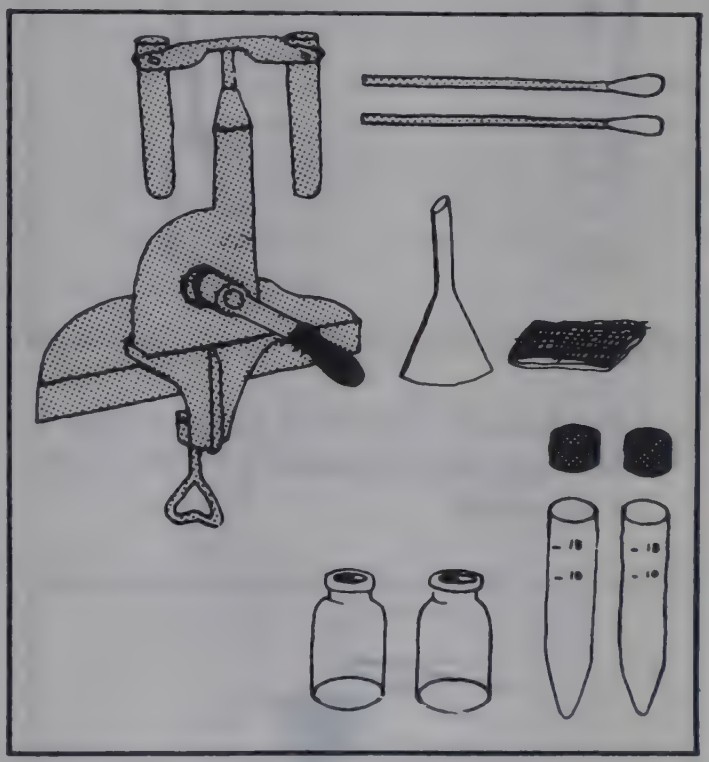
Recommended for:

All eggs and larvae, and especially cysts of protozoa.

Not suitable for:

Motile forms of amoebae and flagellates.

Materials



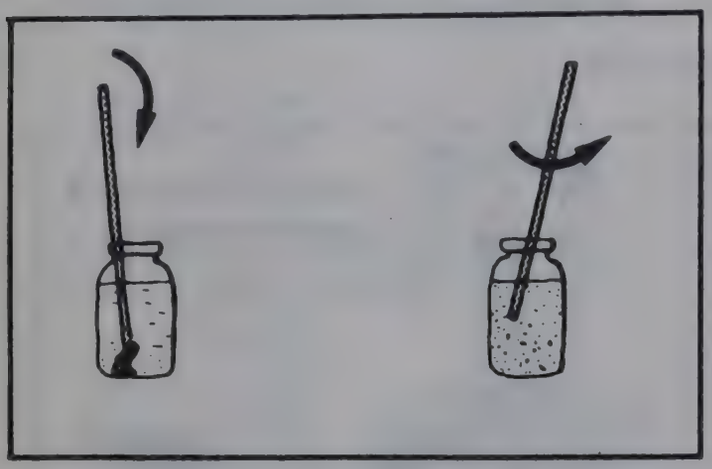
– Centrifuge
– 15 ml conical graduated centrifuge tubes with stoppers
– Wooden applicators
– Empty penicillin bottles
– Funnel
– Gauze
– Graduated cylinder
– Cotton wool swab
– Reagents:
Formaldehyde solution 10%*
Pure ether (if not available, ordinary petrol)

Sodium chloride solution (Reagent No. 25)
Lugol iodine solution (Reagent No. 20)

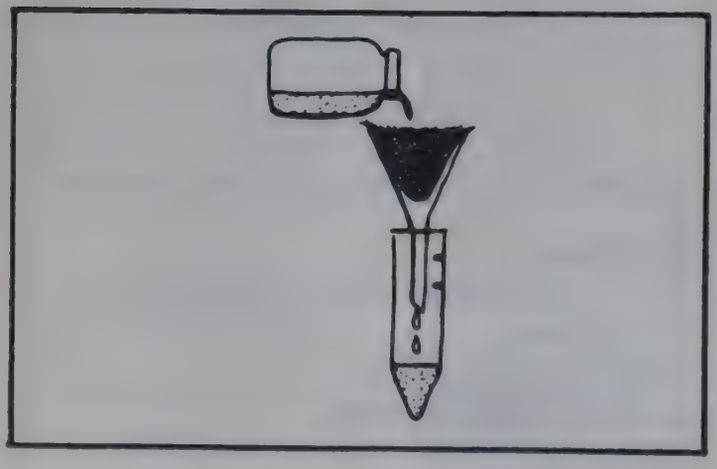
*Commercial formaldehyde solution, at least 37% – 'formalin'	100 ml
Distilled water	300 ml

Method

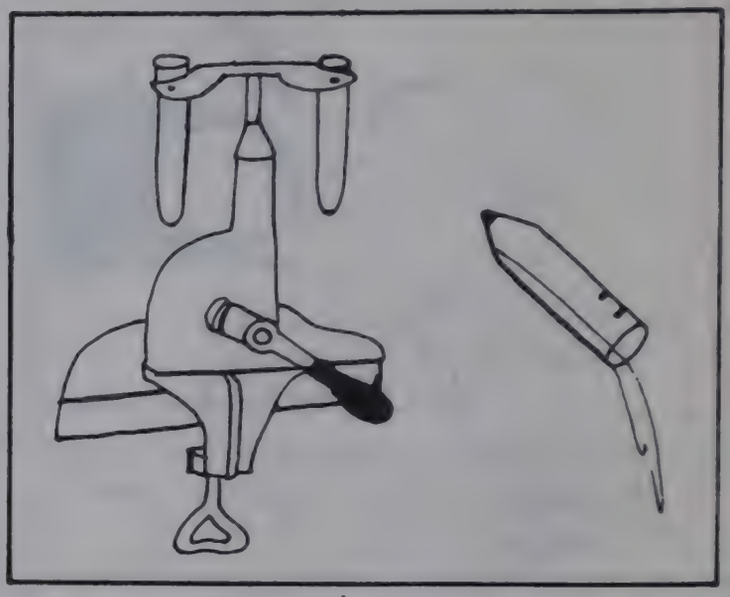
i. Take about 2 ml of stool. Crush and mix it in about 10 ml of sodium chloride solution.



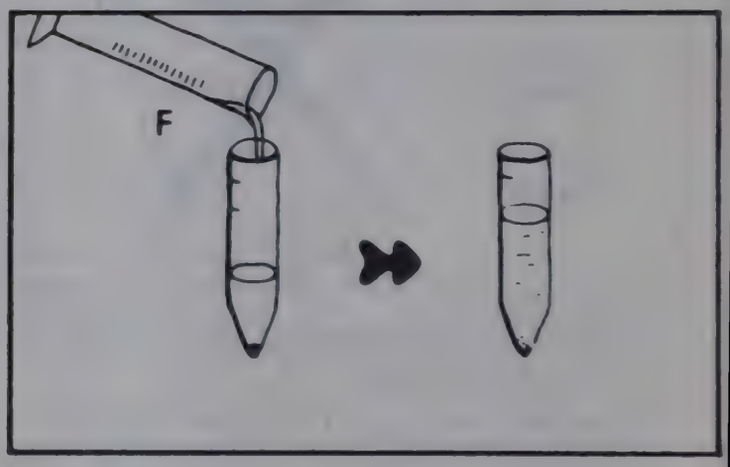
ii. Filter through two layers of gauze into a centrifuge tube graduated with 10 ml and 13 ml marks.



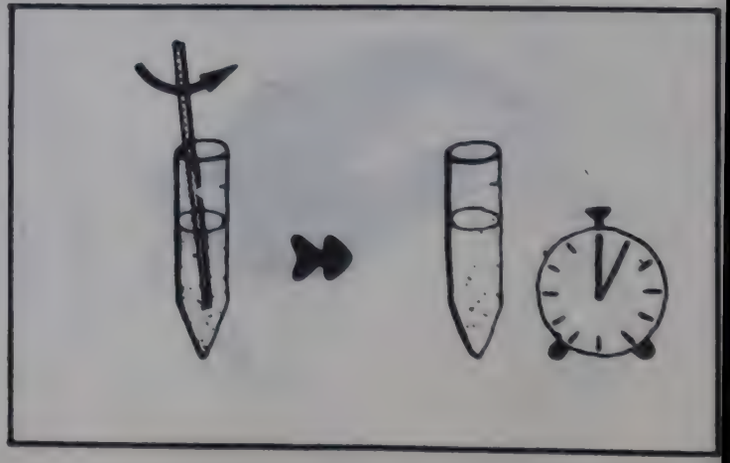
iii. Centrifuge for one minute at medium speed. Pour off the supernatant fluid. If the supernatant fluid is very cloudy, wash the deposit again, i.e. mix it with 10 ml of sodium chloride solution, centrifuge for one minute at medium speed and pour off the supernatant fluid.



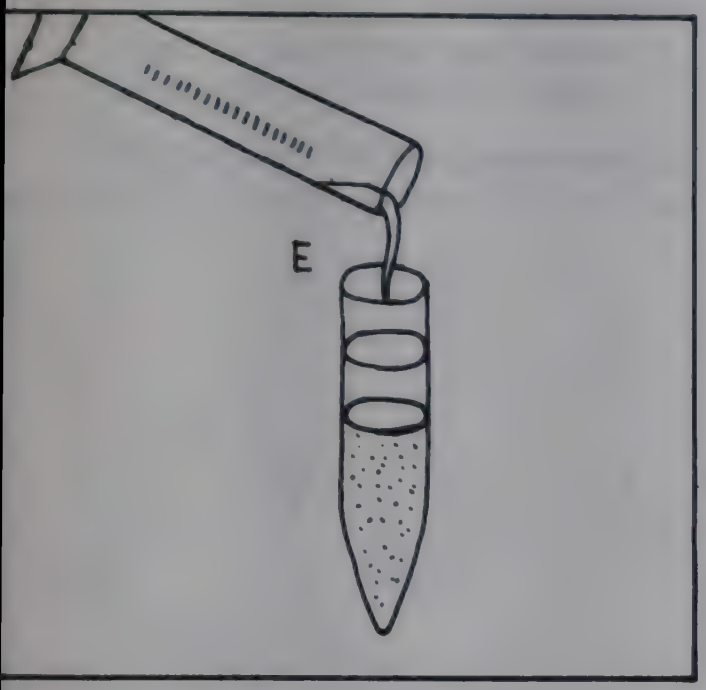
iv. Add 10 ml of formaldehyde solution to the deposit (up to the 10 ml mark).



v. Stir the mixture well and let it stand for 5 minutes.

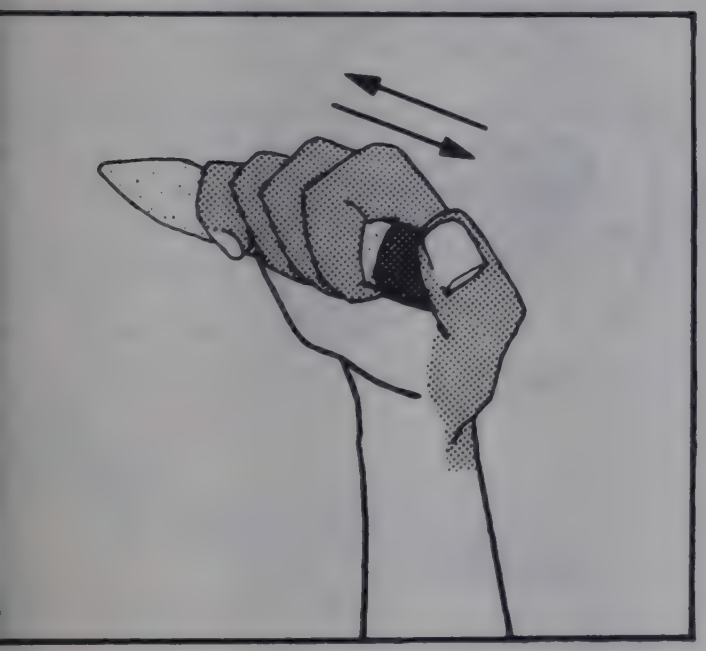


vi. Add 3 ml ether or petrol (up to the 13 ml mark).

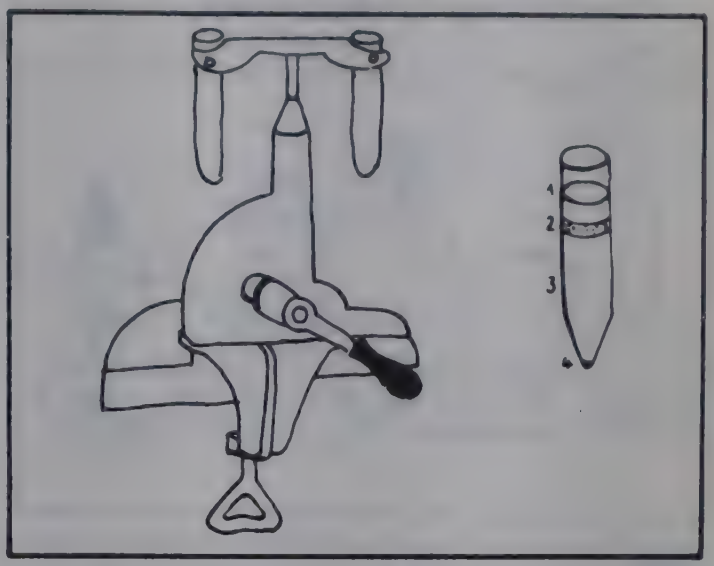


Important: Make sure there is no open flame in the laboratory.

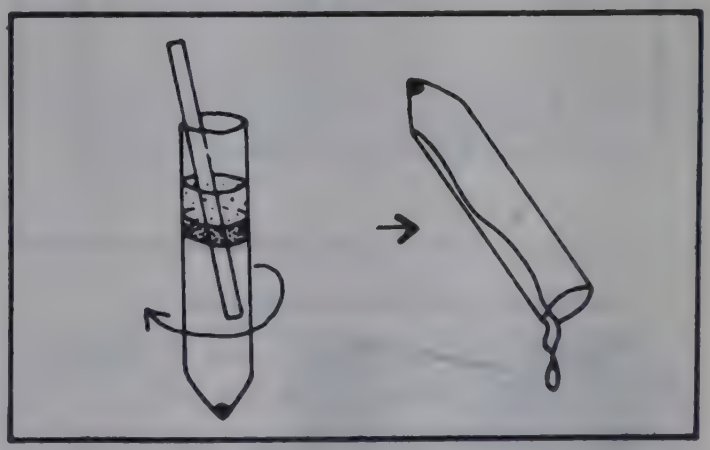
vii. Stopper the tube. Turn it on its side and shake vigorously for 30 seconds.



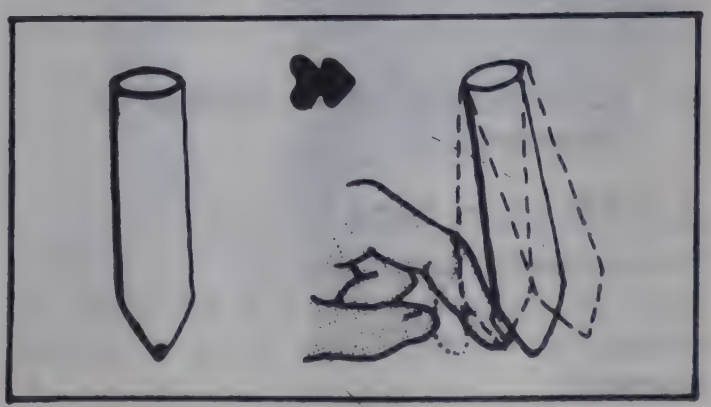
viii. Remove the stopper carefully. Centrifuge for one minute at low speed.
There will be four layers in the tube:
– 1st layer : ether
– 2nd layer : debris
– 3rd layer : formaldehyde solution
– 4th layer : the deposit containing the eggs and cysts of parasites.



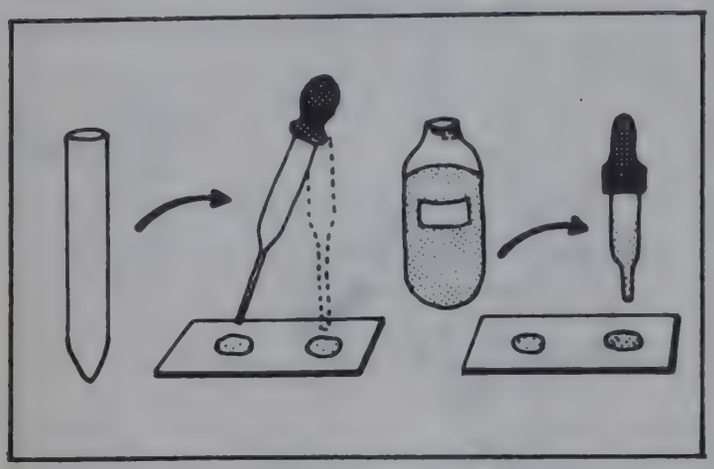
ix. Free the layer of debris by rotating the tip of a wooden applicator between it and the sides of the tube. Tilt the tube and pour off all the supernatant fluid. Use a cotton swab to remove any debris adhering to the side of the tube.



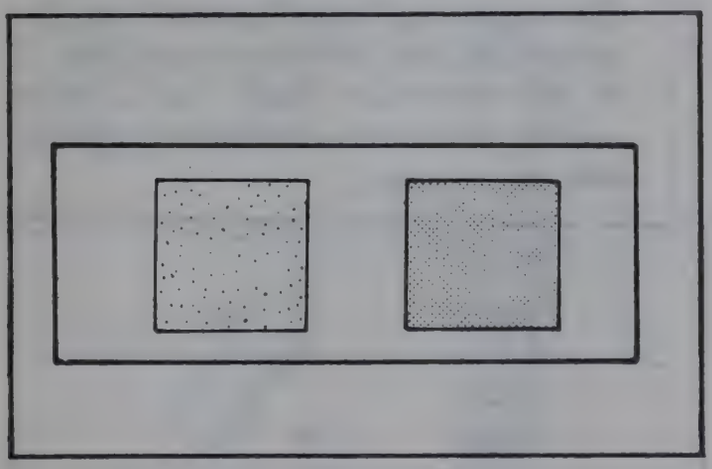
x. Mix the remaining fluid well with the deposit by tapping the tube gently.



xi. Place two drops of the deposit on a slide. Add a small drop of iodine solution to the second drop of deposit only.



xii. Place coverslips over both drops.



Examine under the microscope.

Preparation 1 (unstained): use × 10 and × 40 objectives (eggs, larvae?)

Preparation 2 (stained): use × 40 objective (cysts?)

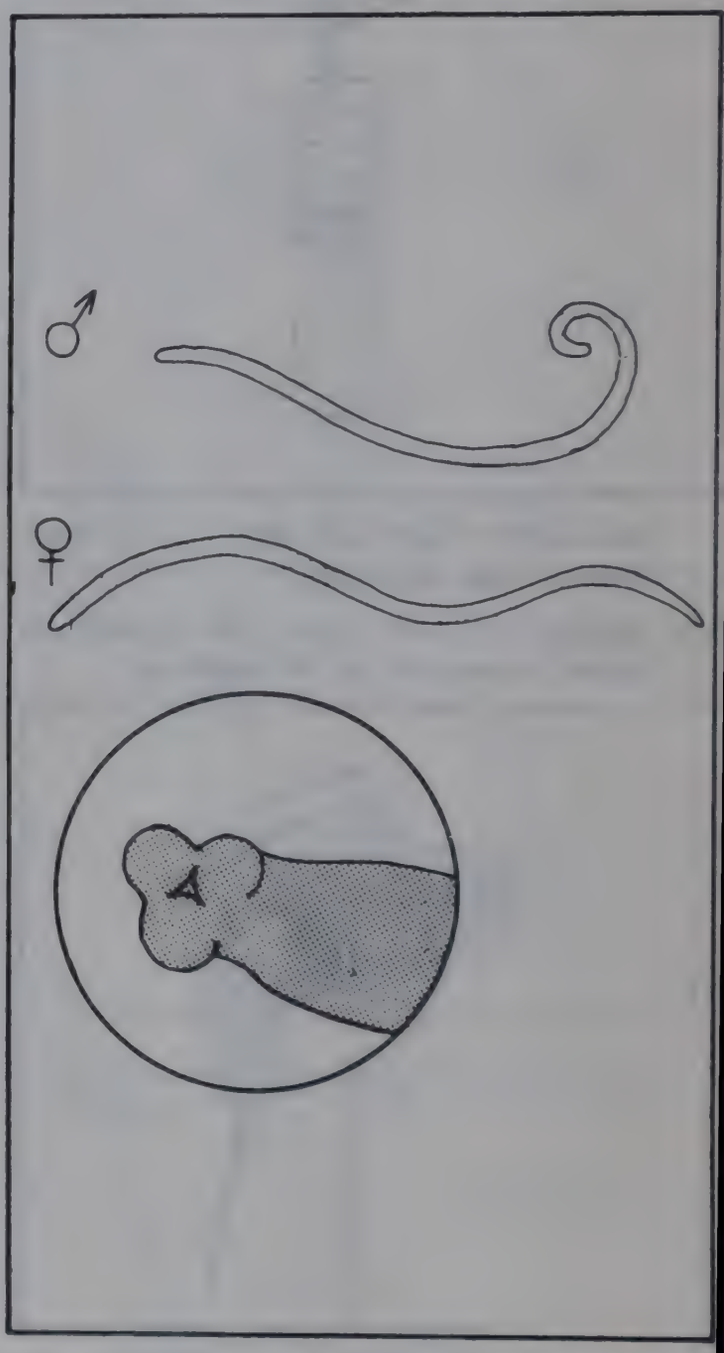
4. Examination for Helminths (Worms)

A. Adult worms found in stools

Worms brought to the laboratory for identification may have been found in the stools, in clothing or bedlinen, or during a surgical operation.

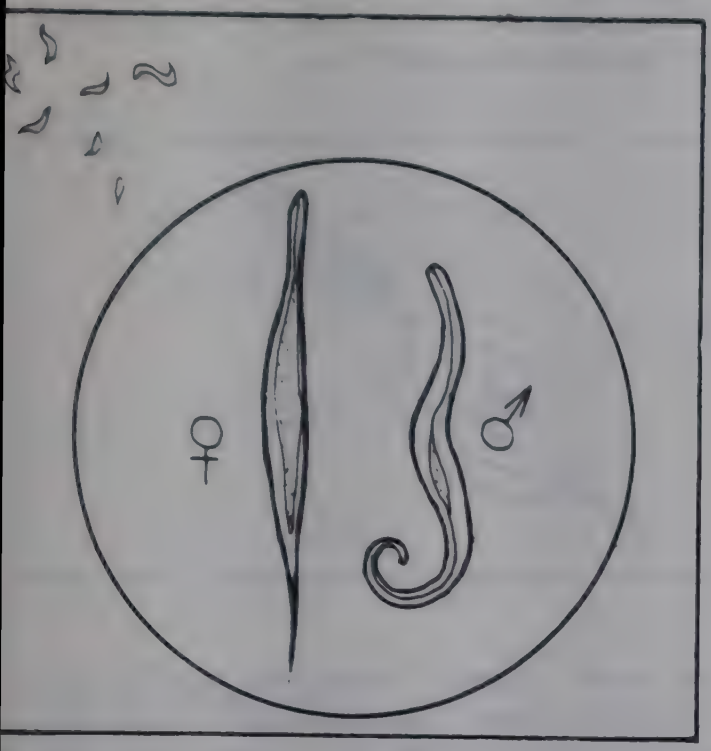
What to examine:
- their length
- their shape
- whether flat, segmented or not
- whether cylindrical (round) or not.

(1) Roundworm (Ascaris lumbricoides)



Colour : pinkish
Thickness : 0.3 – 0.5 cm
Length : male – about 15 cm, with a curled tail
female – 20 – 25 cm, with a straight tail

2) Pinworm (Oxyuris/Enterobius vermicularis)



Colour : white
Length : female – 1 cm, with a very pointed tail
male – 0.5 cm (males are less common)

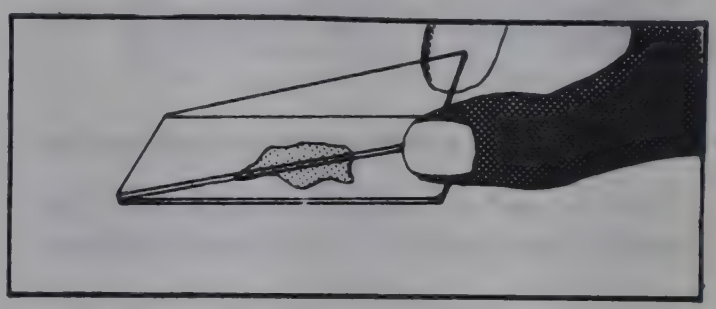
Pinworms are found in large numbers, especially in children's stools, and are motile. They may also be found in the folds of skin around the anus from where they can be collected (See page 97).

3) Tapeworm (Taenia)

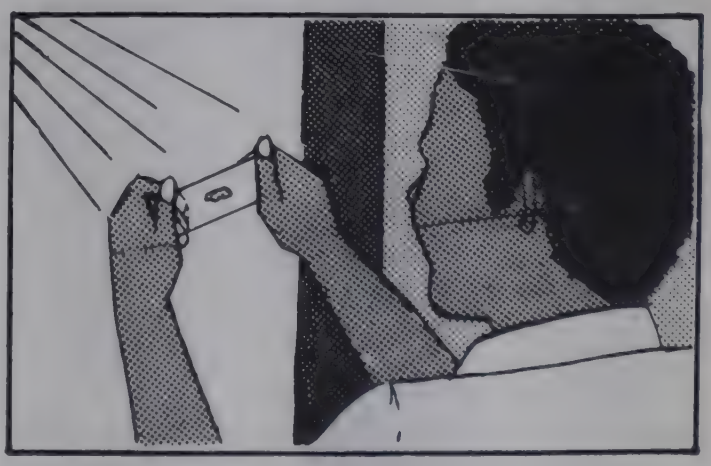
Colour : ivory white or pale blue
Length : total worm 3 – 10 m; but single mature segments (1 – 3 cm long) or fragments of the chain, quite variable in length, are usually presented for examination.

Examination

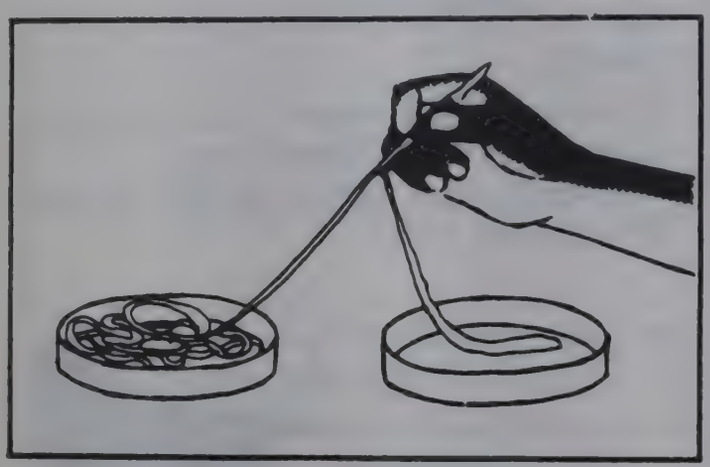
i. Examine a chain of segments to observe the arrangement of the lateral pores.
ii. Examine a single segment gently flattened between two slides.



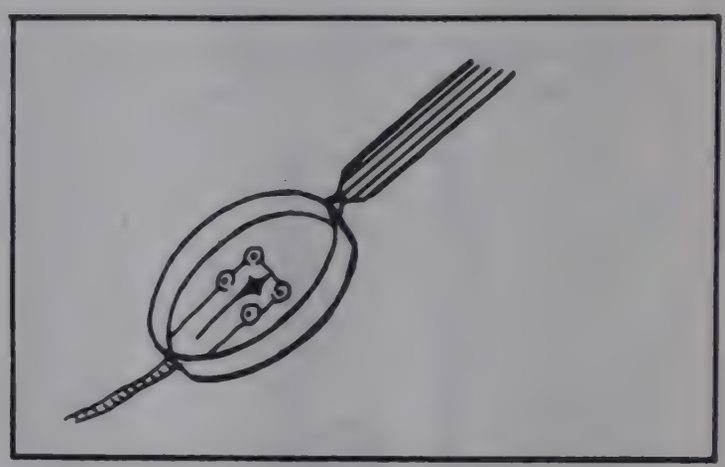
Hold the slide against the light to observe, and count the uterine branches with the naked eye.



iii. To examine the head (scolex):
- Place the whole worm in a Petri dish filled with water (or on a plate)
- Using forceps, transfer the worm little by little into another dish; untangle it, starting with the thicker end.



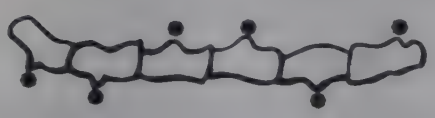
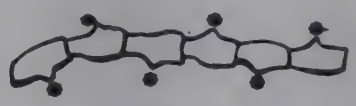
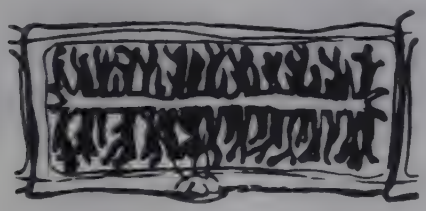
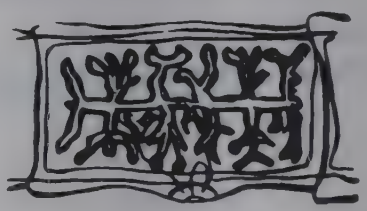
- If at the end of a very narrow section (the neck) a swelling the size of a small pinhead is found, examine it with a magnifying glass or under the microscope (× 10 objective). The head is rarely seen.



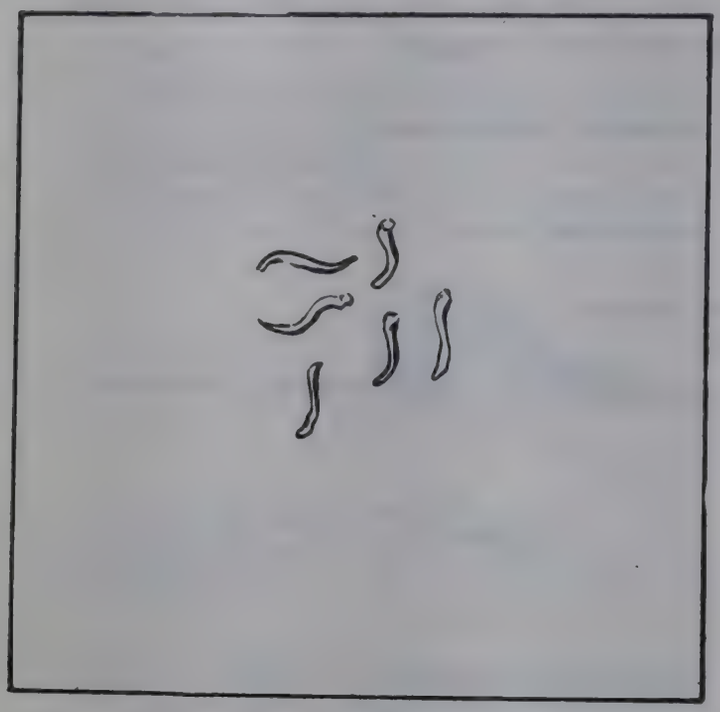
The most common tapeworms are the beef tapeworm (Taenia saginata) and the pork tapeworm (Taenia solium). See Table 8.1 for how to identify them.

TABLE 8.1

How to identify Beef Tapeworm and Pork Tapeworm

Beef tapeworm (Taenia saginata)	*Pork tapeworm (Taenia solium)*
i. Single rectangular segments found in underclothes and in the bed; they pass through the anus independently of the stools. They are part of an intestinal worm 3 – 5 m long.	i. Small chains of 3–4 rectangular segments found in the stools.
ii. Pores arranged in irregular alternation.	ii. Pores generally arranged in regular alternation.
	
iii. Ivory white segments of 1 – 2 cm.	iii. Pale blue segments of 0.5 – 1.5 cm.
iv. About 20 uterine branches.	iv. About 10 uterine branches.
	
v. 4 suckers (2 mm diameter). Very thin neck.	v. 2 crowns of hooklets. 4 suckers (1 mm diameter).
	

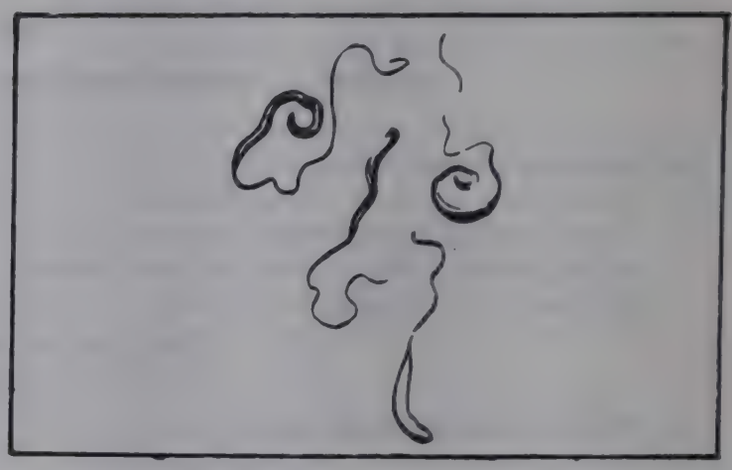
(4) Hookworm (Ankylostoma duodenalis) (Necator americanus)



A small worm like a piece of thread.
Length : 1 – 1.5 cm
Colour :white, or red if it contains blood.

Resembles the pinworm.
Examine the head under the microscope (× 10 objective).

(5) Whipworm (Trichuris trichiura)



A small thin worm.
Length : 3 – 5 cm
Colour : white.

Looks like a tiny whip, one-third being relatively thick and the rest thread-like.

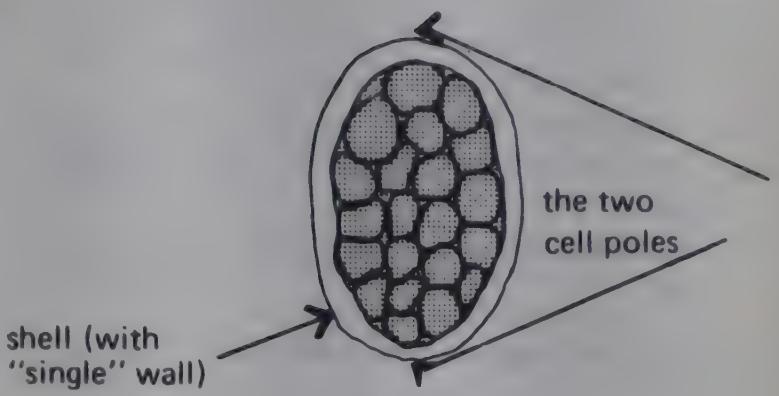
The worm lives in the wall of the caecum or, occasionally, the rectum.

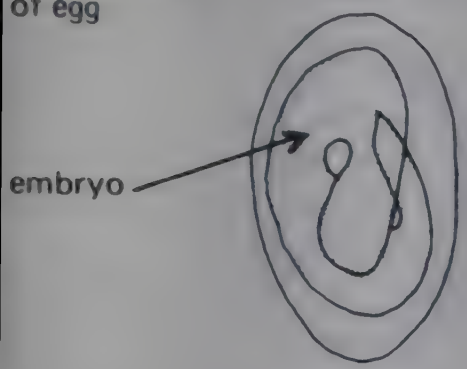
(6) Strongyloides stercoralis

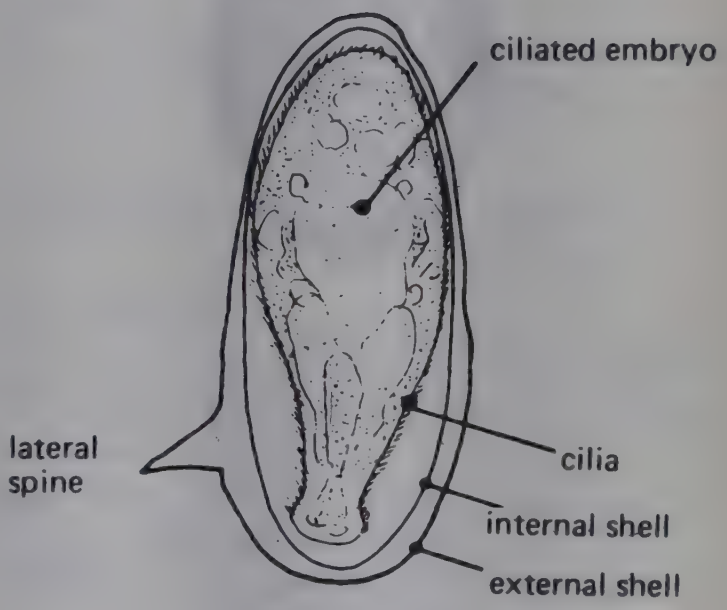
The adult worms are not found in stools. They occur in the lungs and small intestine. This parasite occurs chiefly as larvae in the stools.

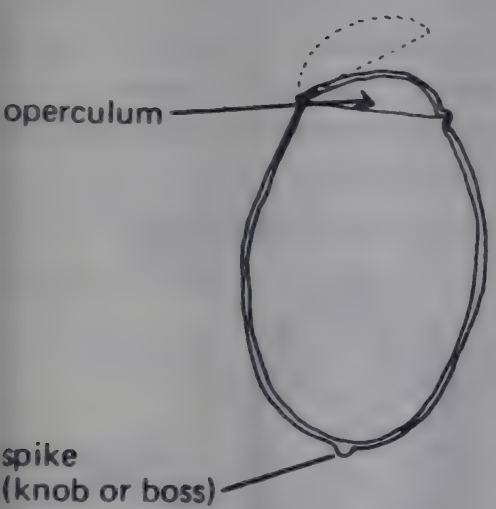
B. Eggs and larvae of worms found in stools

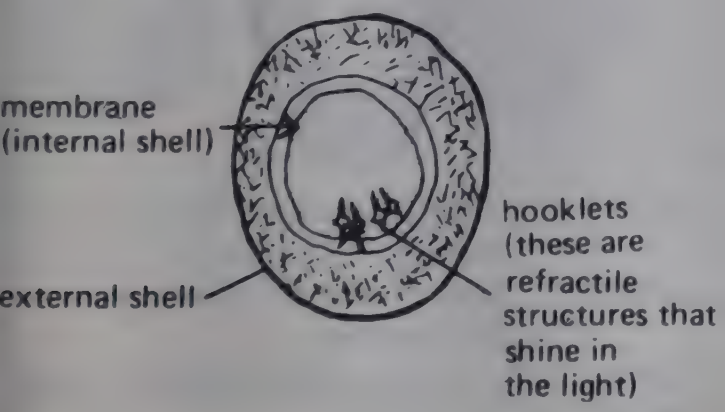
Terms used for identification of eggs:

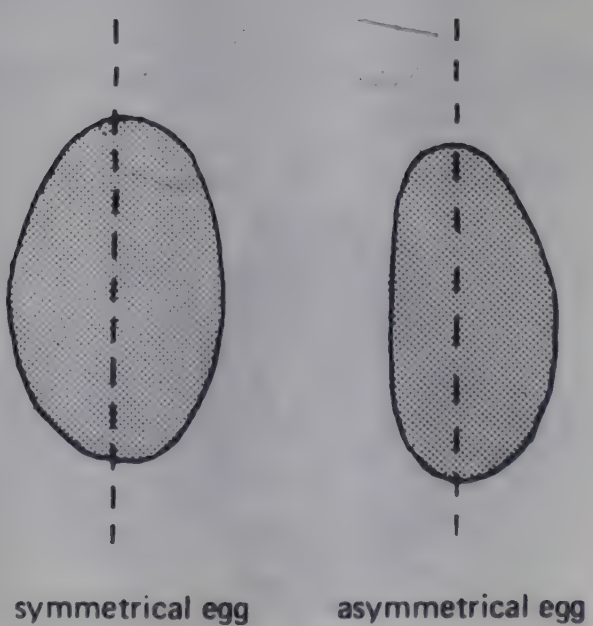








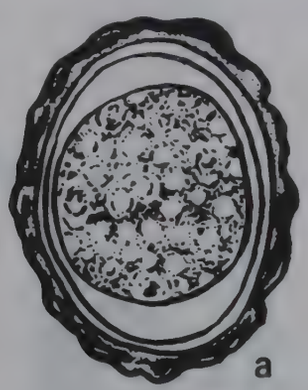




(1) Roundworm (Ascaris lumbricoides)

There are four types of ascaris eggs:
(a) Fertilized egg with double shell:
(b) Unfertilized egg with double shell
(c) Semi-decorticated fertilized egg (less frequent)
(d) Semi-decorticated unfertilized egg (very rare)

(a) Fertilized egg with double shell:



Size : about 70 μ
Shape : oval or sometimes round
Shell : the two shells are distinct:
– the external shell is rough, brown, covered with little lumps (mammillated)
– the internal shell is smooth, thick, colourless.
Colour : the external shell is brown and the contents of the egg are colourless or pale yellow.
Content : a single round granular central mass.

(b) Unfertilized egg with double shell:

Size : about 80 to 90 μ (larger than type (a)
Shape : more elongated (elliptical or irregular)
Shell : the two shells are indistinct:
– the external shell is brown and puffy, with rather jagged lumps
– the internal shell is thin (one or two lines may be visible).
Content : The egg is full of large round very refractile (shiny) granules.

(c) Semi-decorticated fertilized egg:

Similar to type (a) but without the external shell.
Shell : single, smooth, thick and colourless (or very pale yellow)
Content : a single round colourless granular central mass.

(d) Semi-decorticated unfertilized egg:

Shell : a single smooth thin colourless shell (double line)
Content : large roundish colourless refractile granules

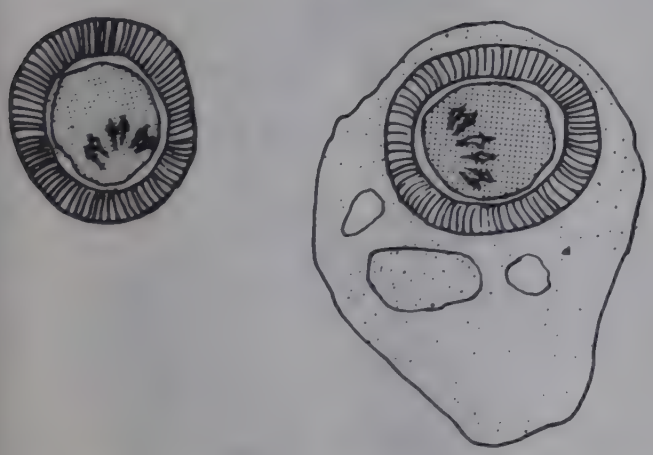
Caution: Do not confuse type (d) with the egg of Ankylostoma duodenale.

(2) Pinworm (Enterobius vermicularis)

Size : 50 – 60 μ
Shape : oval but clearly asymmetrical (flattened on one side, rounded on the other)
Shell : smooth and thin, but a double line is visible
Content : either (a) a small, granular mass in the shape of an irregular oval
or (b) the embryo of the worm, a small curled up larva
Colour : transparent, colourless.

The egg is usually more easily found in the folds of skin around the anus. For collection of pinworm eggs see page 97.

(3) Tapeworm (a) Taenia saginata
(b) Taenia solium

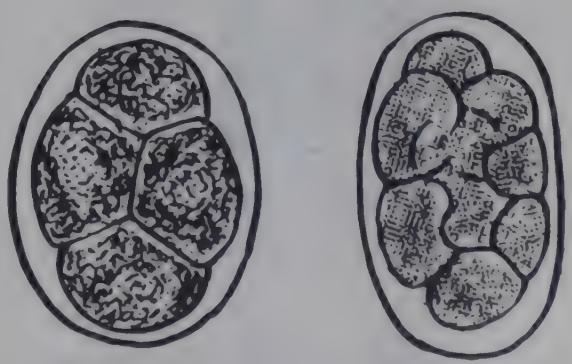


The eggs of these two tapeworms are practically identical. They may be found in stools and T.saginata eggs can also be collected from the skin around the anus (see page 97).

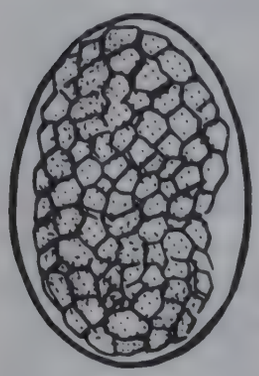
Size : 30 – 40 μ
Shape : round
Shell : very thick, smooth, with transverse lines (reduce illumination)
Colour : – shell: dark yellowish-brown
– content: light yellowish-grey
Content : a round granular mass enclosed by a fine membrane, with 3 × 2 refractile lancet-shaped hooklets (adjust the focus)
External sac : sometimes the egg is enclosed in a floating transparent sac.

(4) Hookworm (a) Ankylostoma duodenale
(b) Necator americanus

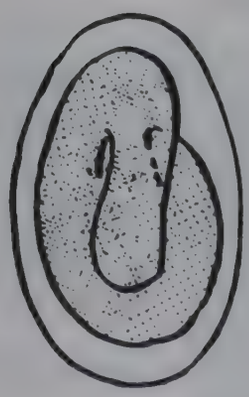
(a) A. duodenale:
Size : 50 – 60 μ
Shape : oval with rounded slightly flattened poles (one pole often more flattened than the other)
Shell : very thin; appears as a black line
Colour : the cells inside are pale grey (iodine solution turns them dark brown)
Content : Varies according to the degree of maturity –
Fresh stools: 4, 8 or 16 grey granular cells, clear but not refractile (blastomeres).



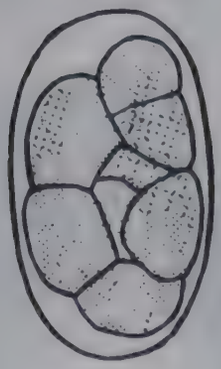
Stools a few hours old: A uniform mass of many small grey granular cells.



Stools 12 to 48 hours old: The whole of the egg is filled by a small larva (the future worm) wrapped around itself. The egg is 'embryonate'.



(b) N. americanus:



The egg is almost identical with that of A. duodenale.

Size : a little longer (70 μ)
Shape : poles more flattened
Content : always contains at least 8 cells (never 4 like A. duodenale) in fresh stools.

(5) Whipworm (Trichuris trichiura)

Size : 50 μ
Shape : barrel-shaped
Shell : fairly thick and smooth, with two layers
Colour : shell orange, content yellow
Other features : a rounded transparent plug at each pole
Content : a uniform granular mass (sometimes divided in old stools).

Note: It is important to specify whether there are many or few whipworm eggs present.

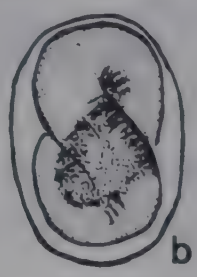
(6) Strongyloides stercoralis

(a) Larvae:

The larvae are highly motile in the stools.

Size : 200 – 300 μ long, 15 μ thick
Tail : moderately tapered
Mouth : short
Digestive tube : easily visible, with an oesophagus (O) with two swellings at one end and an anal pore (A) at the other
Genital primordium : a rounded clear space near the middle of the larva (E).

(b) Eggs:



The eggs are seldom seen in formed stools because they hatch before evacuation to produce the larvae described above. They may, however, be found in liquid stools.
The eggs are very similar to those of A. duodenale.

Size : 50 μ (slightly smaller)
Shape : similar to that of A. duodenale
Shell : similar to that of A. duodenale
Colour : similar to that of A. duodenale
Content : a thick larva curled around itself one or more times and sometimes motile.

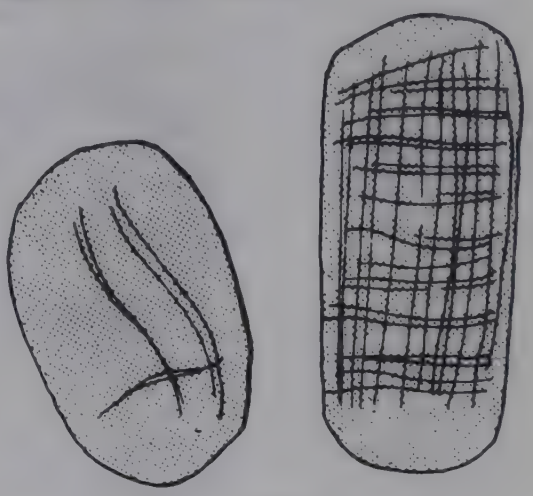
Not to be mistaken for eggs

(1) Starch granules from plants

Size : 50 – 100 μ
Shape : round or oval or elongated, but the outline is always irregular, with rough indentations
Shell : thick in places, very irregular, with cracks
Colour : whitish or greyish-yellow; iodine solution turns it to violet
Content : masses of starch packed closely.

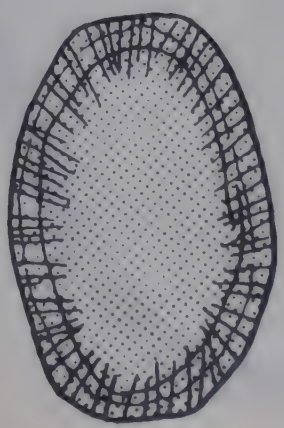
These granules are the residue of starchy foods, e.g. potatoes, beans, yams, etc.

(2) Digested meat fibres



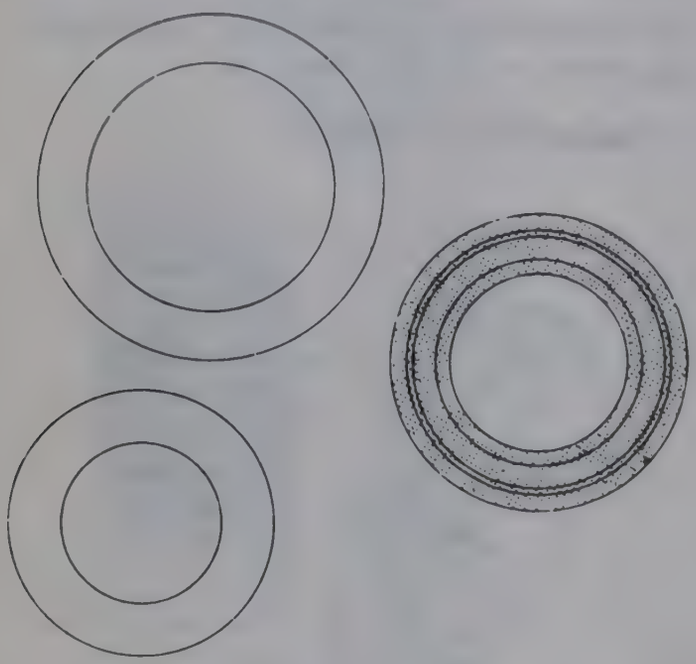
Size : 100 – 200 μ
Shape : oval or rectangular with rounded corners
Colour : yellow
Content : Transparent with no granulations or lines (or residual lines where meat is not properly digested).

(3) Soaps



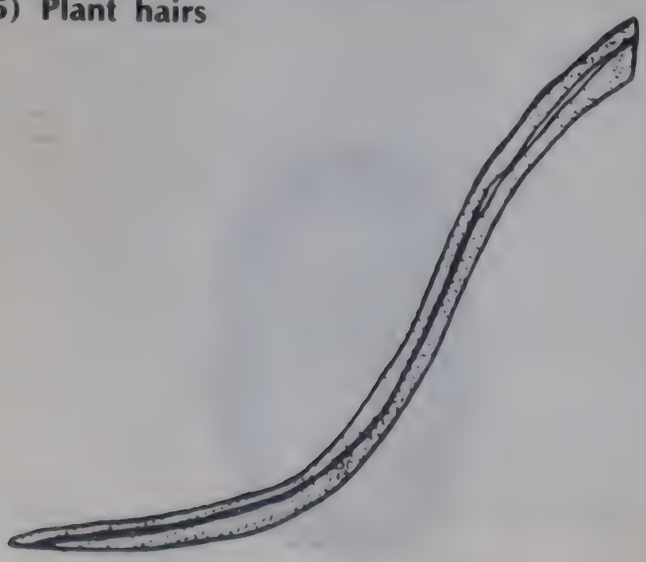
Size : 20 – 100 μ
Shape : round, oval or irregular (like a section of a tree trunk)
Colour : brownish-yellow or colourless
Content : lines radiating from the centre and visible near the rim; nothing in centre.

(4) Air bubbles - oil droplets



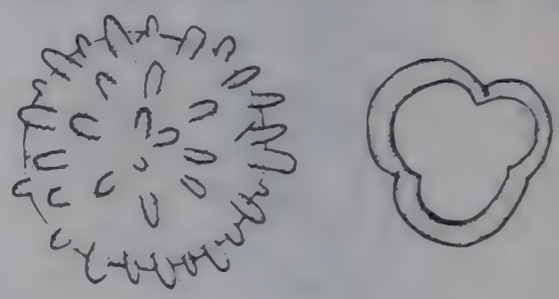
Size : variable (can be any size)
Shape : perfectly round
False shell : a circular ring, very refractile (several layers in the case of oil)
Content : none

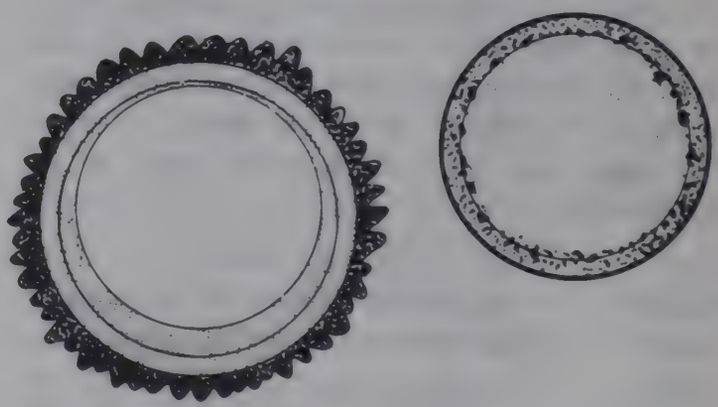
(5) Plant hairs



Size : very variable (50 – 300 μ)
Shape : rather rigid, often curved; wide and clean-cut at one end, tapered at the other.
Colour : pale yellow
Content : a narrow empty central canal between two transparent refractile layers.

(6) Pollen grains and fungus spores





Vary widely according the the area and the diet. Their peculiar and distinctive geometrical shapes and other features (saw-like or rounded projections, etc.) help to distinguish them from the eggs of parasites.

5. Examination for Protozoa

Protozoa are microorganisms consisting of a single cell. They may be found in stools in their motile form (trophozoites) or as cysts.
Protozoa include:

Amoebae – without flagella or cilia
Flagellates – with flagella
Ciliates – with cilia

Some intestinal protozoa are pathogenic; others are less so or are harmless.

For instance:

(1) Amoebae

(a) Entamoeba histolytica: This amoeba, which may cause dysentery or abscesses, is the only amoeba that is commonly pathogenic in man.
(b) Entamoeba coli: Non-pathogenic, but very common.

(2) Flagellates

(a) Giardia lamblia: Pathogenic.
(b) Trichomonis hominis: Non-pathogenic.

(3) Ciliates

(a) Balantidium coli: Pathogenic.

The chief problem for the laboratory, therefore is:

– the precise identification of: E.histolytica
G.lamblia
B.coli

A. Motile vegetative forms

The trophozoites of protozoa are motile:
– either because of slow movements of the cell (amoebae)
– or because they have rapidly moving flagella (long whip-like threads) or cilia (numerous short hairs).

Protozoa in motile form are chiefly found in:
– fluid stools
– stools containing mucus
– soft unformed stools.

Preparation of slides for examination

(1) Examine only fresh stools (maximum 1 hour old). Amoebae become non-motile very quickly.

(2) If you receive a number of stool specimens at once, begin by examining the most fluid specimens containing mucus.

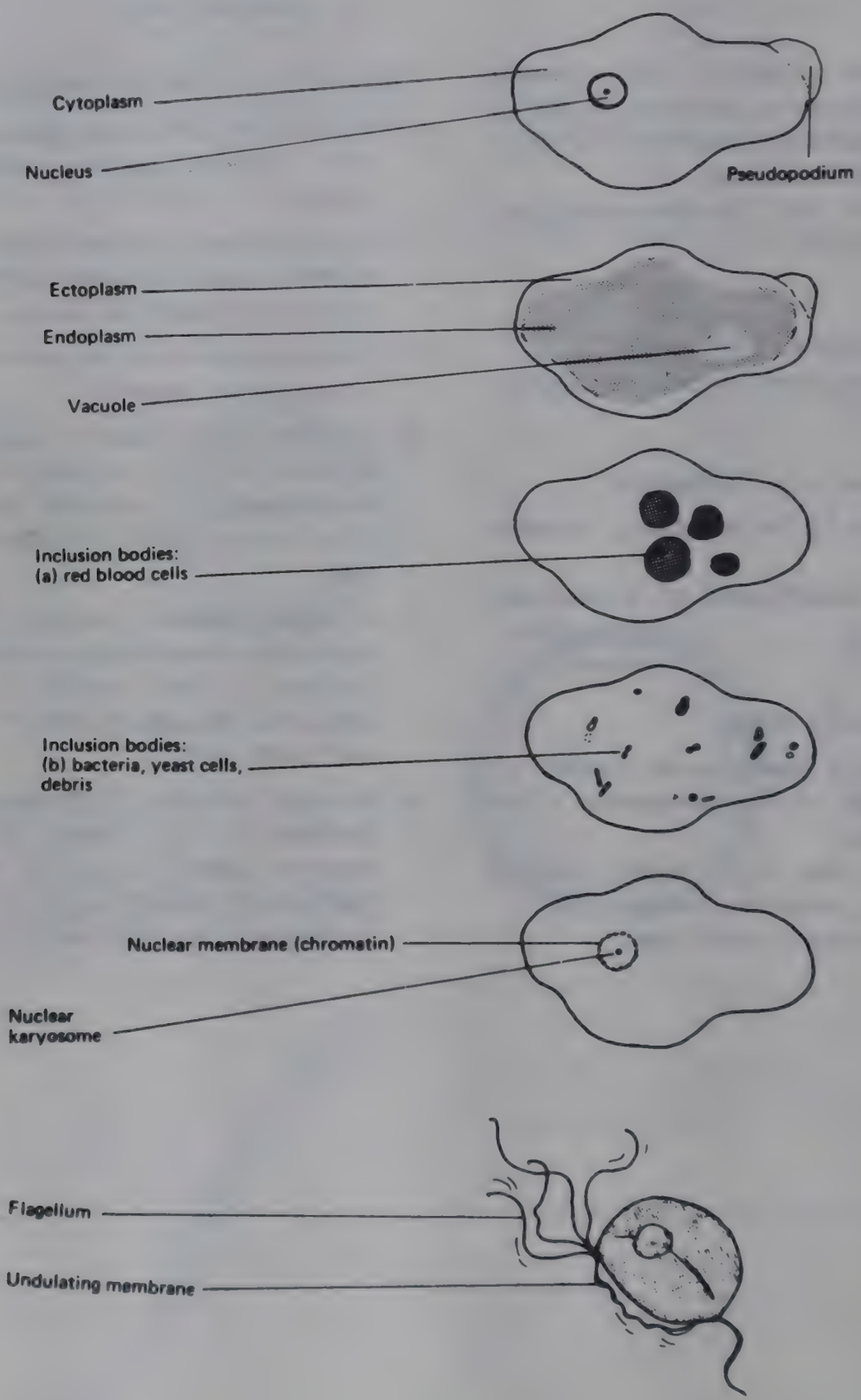
(3) Take a portion from the outside of the stool where mucus appears.

(4) Examine in a sodium chloride solution (Reagent No. 25), warming slightly in low temperatures, or examine directly if the stools are very liquid. Use the × 40 objective.

(5) In iodine solution (Reagent No. 19) the trophozoite forms become non-motile. The nucleus is clearly stained but it may be difficult to distinguish between trophozoite and cystic forms.

(6) If a drop of eosin, 20 g/l solution in saline (Reagent No. 12) is added, the whole field becomes stained except for the protozoa (particularly amoebae), which remain colourless and are thus easily recognized.

Some features useful for the recognition of motile forms of intestinal protozoa:



Amoebae

(1) Entamoeba histolytica (dysentery amoeba)

Size : varies from 12 to 35 μ (usually as long as 3 or 4 red blood cells)

Shape : when moving, elongated and changing; when not moving, round

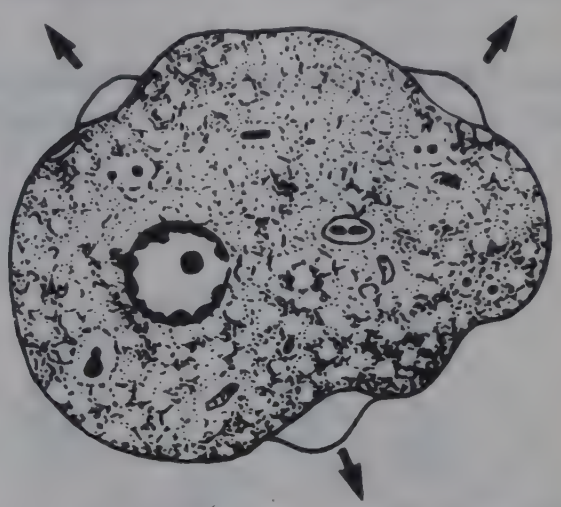
Motility : moves in one direction; a pseudopodium pushes forward and the endoplasm flows quite rapidly into it

Cytoplasm : the ectoplasm is transparent, quite different from the fine granular texture of the endoplasm (greyish shot with yellowish-green), which may contain vacuoles

Inclusion bodies : red blood cells (1 to 20 of different sizes)

Nucleus : not visible in the motile form, but when stained with iodine solution clearly seen to have a regular membrane and a small dense central karyosome (a black dot).

(2) Entamoeba coli (non-pathogenic)



Size : 20 – 40 μ (usually bigger than E.histolytica)

Shape : oval or elongated, rather irregular

Motility : often non-motile or moving very slowly, putting out blunt pseudopodia in all directions, gropingly

Cytoplasm : both the ectoplasm and the endoplasm granular and difficult to differentiate

Inclusion bodies : numerous and varied (bacteria, yeast cells, debris of all sorts), *but never red blood cells*

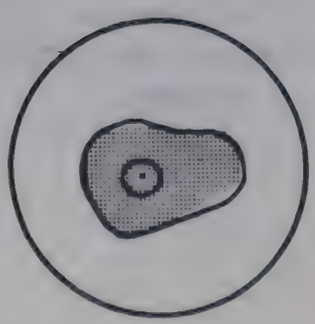
Nucleus : visible in the fresh state, without staining. The membrane is irregular and granular (like a bead necklace), the karyosome large and eccentric.

A comparison of E. histolytica and E. coli is given below:

Characteristic	*E. histolytica*	*E. coli*
Motion	In a definite direction.	Haphazard.
Motility	Fairly motile.	Non-motile or barely motile.
Ectoplasm	Transparent, quite different from the endoplasm.	Little or no differentiation from the endoplasm.
Inclusion bodies	Red blood cells.	No red blood cells.
Nucleus (fresh state)	Invisible.	Visible (nuclear membrane like a bead necklace).
Nucleus (after staining with iodine solution)	Regular membrane. Small dense central karyosome.	Irregular membrane. Large eccentric karyosome.

Entamoeba histolytica

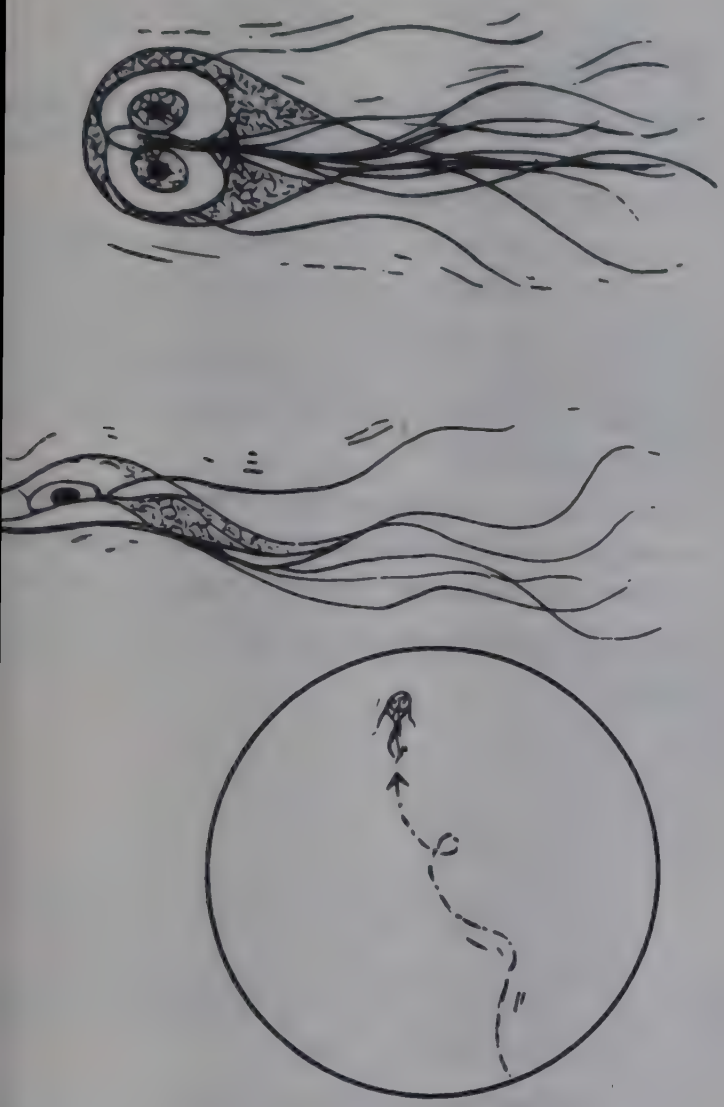
Entamoeba coli



(stained with iodine solution)

lagellates

) Giardia lamblia (the longest flagellate)



ze : 10 to 18 μ (size of 2 red blood cells)

ape : rather elongated:
front view: like a pear
side view: spoon-shaped

otility : either moves forward in little rapid jerks in a definite direction, sometimes turning in a loop (fluid stools), or hardly motile

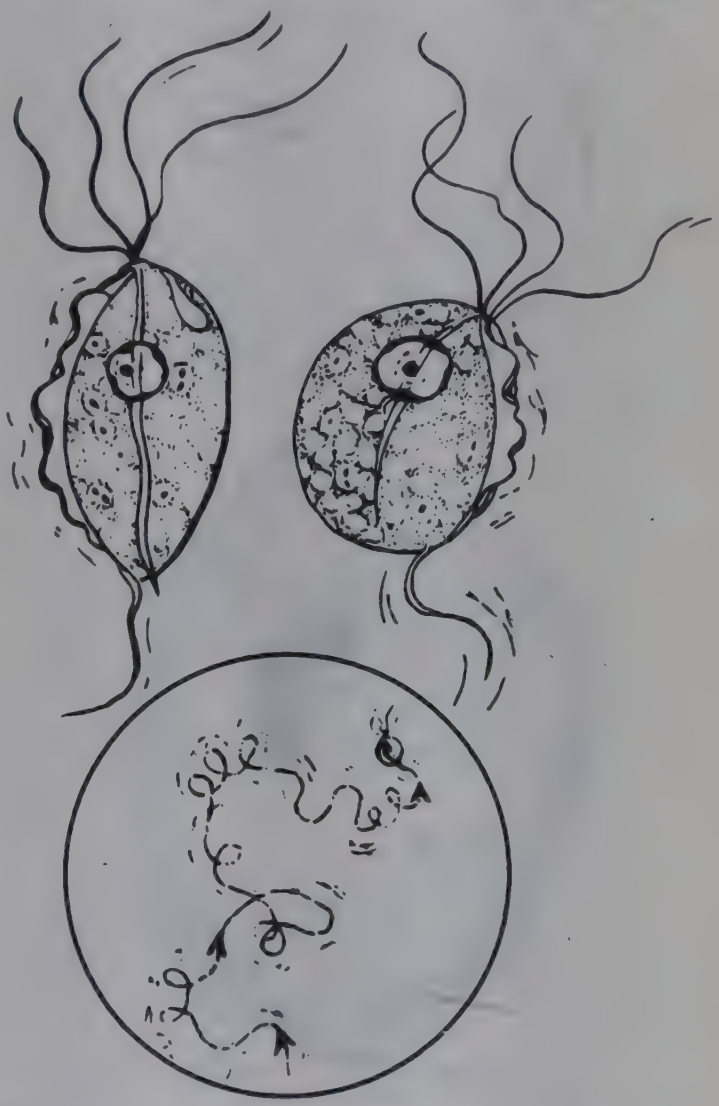
ntent : 2 large oval nuclei, faintly visible.

ote:The characteristic movement is seen only fresh liquid stools.

kes of mucus in fluid stools often contain clusters of G. lambiia in large numbers.

The vegetative and cystic forms of G. lamblia are often found together in soft stools.

(2) Trichomonas hominis (non-pathogenic)



Size : 10 – 15 μ (slightly smaller than G. lamblia

Shape : oval with two pointed poles

Motility : whirls and turns in all directions seeming to vibrate

Undulating membrane : present on one side only; extremely motile (a rapid wavy movement)

Nucleus : one nucleus, difficult to see

Flagella : usually 4.

Trichomonas is the most resistant flagellate. It remains motile even in old stools.

Ciliates

(1) Balantidium coli (rare)

Size : very large – 50 μ (often as big as or bigger than a roundworm egg)

Shape : oval, with one pole more rounded than the other, transparent

Cilia : covered with many small cilia, which move with rapid strokes

Motility : moves very rapidly in stools, crossing the field in a definite direction and sometimes turning in circles

Nucleus : a large kidney-shaped nucleus next to a small round nucleus

'Mouth' : the cytostome: a sort of mouth that contracts and expands, drawing in debris (M).

B. Cysts

Cysts are the small round non-motile resistant forms of certain intestinal protozoa. Cysts may have one or several nuclei.

It is important to be able to find and recognize cysts of E. histolytica, G. lamblia and B. coli, although their presence in the stools is of less immediate significance than the presence of the vegetative forms. Healthy persons may be carriers of cysts.

The cyst is the infective form. Carriers of cysts, therefore, are a public health hazard. Detection of cysts may also be of epidemiological value.

Cysts are usually found in both soft and hard stools.

Slide examination

(1) *Preparation in sodium chloride solution* (Reagent No. 25): Cysts can be seen as transparent refractile globules standing out clearly against the grey background. They have well-defined shells.

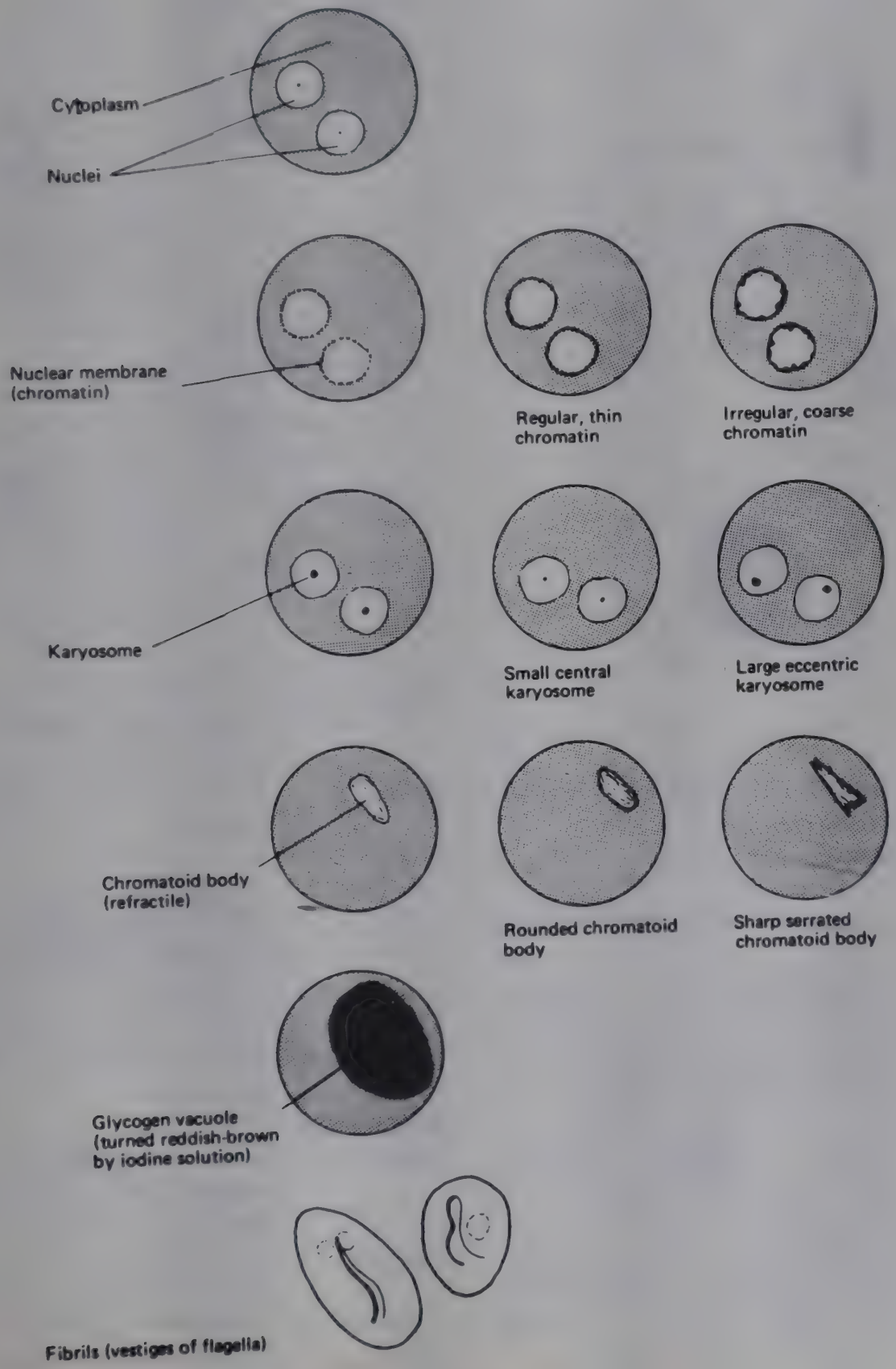
(2) *Preparation in iodine solution* (Reagent No. 19 diluted 5 times): The nuclei become stained. Examine under the × 40 objective.

(3) *Counting the nuclei*: Turn the fine adjustment screw of the microscope.

(4) *Identification*: It is never enough to recognize a single cyst. Identification depends on the observation of several in succession.

(5) *Concentration*: If necessary, use the formaldehyde-ether concentration method (see page 99) to examine a larger number of cysts for surer identification.
Cysts of several different species may be found in the same stool specimen.

ome features useful for the recognition of cysts of intestinal protozoa:

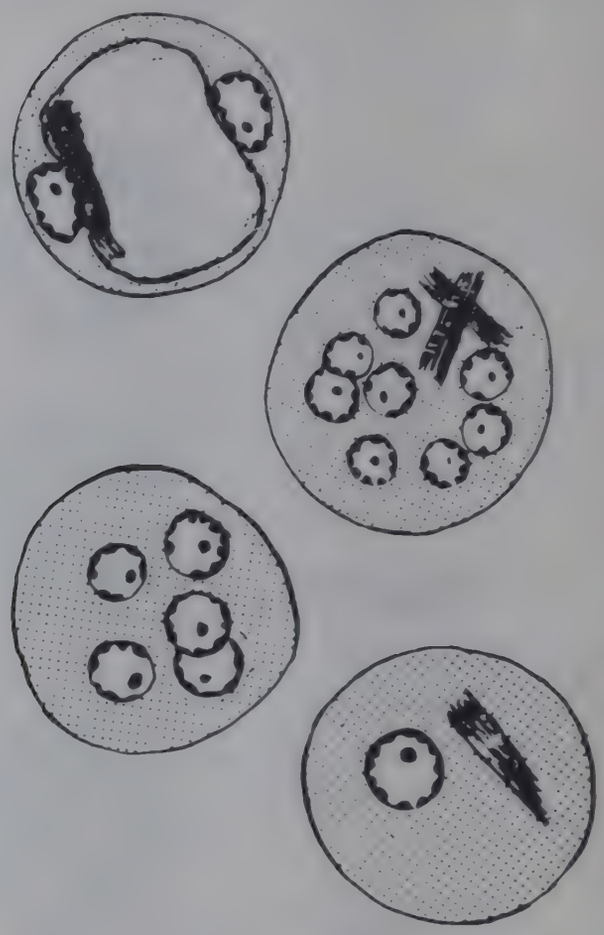


Amoebae

(1) Entamoeba histolytica

Size : 12–15 μ ($1^1/_2$ –2 red blood cells)
Shape : round
Nuclei : 1 – 4 nuclei:
– membrane thin, regular, circular
– karyosome small, compact, central (like a black dot)
Cytoplasm : (iodine solution) yellowish-grey and granular; looks 'dirty'
Chromatoid bodies : oblong,, rounded at ends (sausage-shaped); not found in all cysts
Vacuole : sometimes a large glycogen vacuole (stained reddish-brown by iodine solution) in young cysts with 1 or 2 nuclei.

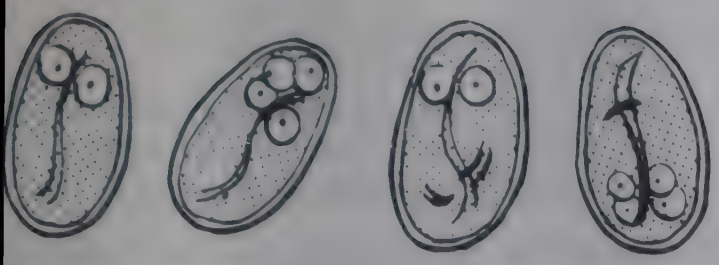
(2) Entamoeba coli



Size : 12 – 20 μ (2 – $2^1/_2$ red blood cells); a little larger than the cyst of E. histolytica
Shape : round or slightly oval, sometimes irregular
Nuclei : 1 – 8 nuclei:
– membrane irregular, thick in parts, not a perfect circle
– karyosome large, diffuse, often eccentric
Cytoplasm : (iodine solution) pale yellow, bright (as compared with E. histolytica)
Chromatoid bodies : sharp or jagged ends (dagger-shaped or needle-shaped); not found in all cysts
Vacuole : sometimes a very large vacuole (stained brownish-red by iodine solution) compressing two nuclei, one at either pole.

Flagellates and ciliates

(1) Giardia lamblia



Size : 8–12 μ
Shape : oval, one pole more rounded than the other
Shell : often appears to be a thick shell with a double wall; in fact the second wall is the membrane of the cytoplasm
Nuclei : 2 – 4 oval nuclei:
– membrane very fine
– karyosome small, central, faintly coloured
Cytoplasm : clear, refractile when unstained, pale yellowish-green or bluish in iodine solution
Fibril : refractile, hair-like line, folded in two or S-shaped, placed lengthwise in the centre of the cyst (adjust microscope)

(2) Balantidium coli

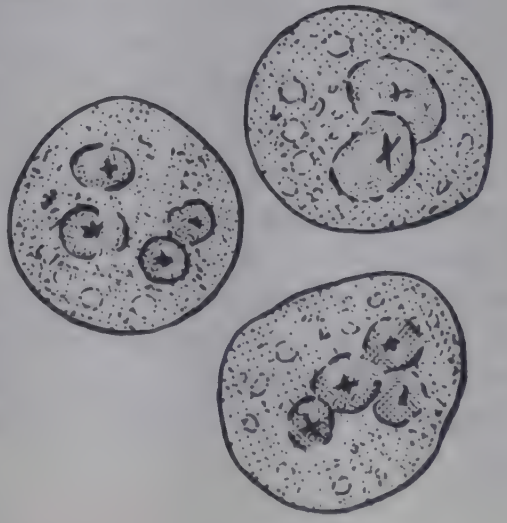
Size : very large cyst, 50–70 μ)the size of a roundworm egg)
Shape : round
Shell : thin double-wall
Nuclei : 1 large kidney-shaped nucleus
1 small nucleus like a thick dot beside the large nucleus
Cytoplasm : granular, greenish, filled with inclusion bodies

Not to be mistaken for cysts

(1) Yeasts

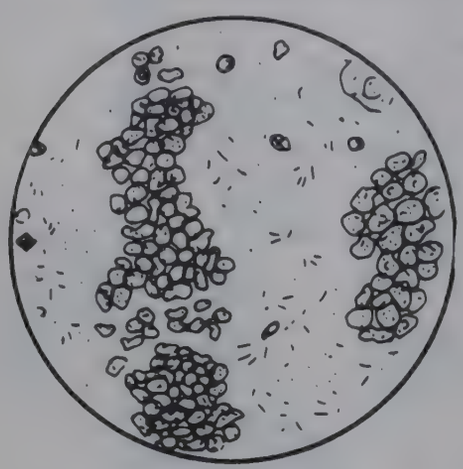
Size : Small (5–8 μ)
Shape : oval, often with buds
Content : Often an eccentric cluster of 3 – 6 small granules
Colour : (iodine solution) brownish red

(2) Leucocytes



Size : 10–20 μ
Shape : round or slightly elongated, with an irregular outline
Content : refractile cytoplasm, clear and granular with tiny vacuoles
Nucleus : indistinct, sometimes with a star-shaped 'false karyosome'.

(3) Pus



Pus can be seen by the naked eye as opaque, greyish streaks (not transparent like mucus). Under the microscope it appears as a mass of more or less degenerate leucocytes.
Report its presence.

6. Recording the Results of Stool Examination

In reporting on the stool specimen, note:

(1) The appearance of the stool (quantity, consistency, colour and presence of abnormal features.

(2) The presence or absence of parasites, i.e.
- type of worms or protozoa seen, e.g. A. duodenale, E. histolytica, etc.
- stage of parasite, e.g. egg, larva, vegetative form, worm segment, etc. When describing E. histolytica, always specify whether it contains ingested red blood cells.
- quantity, i.e.
  - no ova or parasites seen
  - occasional (1 – 2 per slide)
  - a few (3 – 5)
  - moderate number (6 – 12)
  - many (more than 12)
- mention whether the result was obtained by direct examination or by using a concentration method (state which).

Some examples of reports of stool examination are given below:

Smt A. B.: Soft, unformed stools.
No ova or parasites seen by direct examination or after concentration (formaldehyde-ether).

Shri M. N.: Liquid stools showing blood-stained mucus.
Direct examination: Moderate number of vegetative forms of E. histolytica containing ingested RBCs. A few eggs of hookworm.

Shri X. Y.: Stool specimen received very small and dried-up.
Direct examination: no ova or parasites seen.
State of specimen made examination for vegetative forms of protozoa impossible.

7. Despatch of Stool Specimens

It is often necessary to send stools elsewhere for bacteriological culture, e.g.
- for the detection of cholera vibrios
- for the detection of other bacteria causing dysentery (Salmonella, Shigella, etc.)

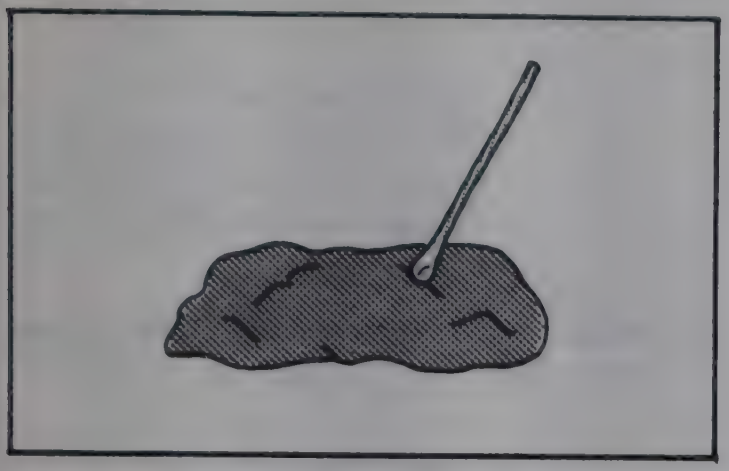
This can be done as follows:

(1) Using Cary-Blair Transport Medium (Reagent No.8)

Cary-Blair transport medium will preserve many kinds of enteric bacteria (cholera vibrios, salmonella, shigella, etc.) for up to 4 weeks. The uninoculated medium may be stored at room temperature for 8 – 12 weeks before use, if in a sealed bottle.

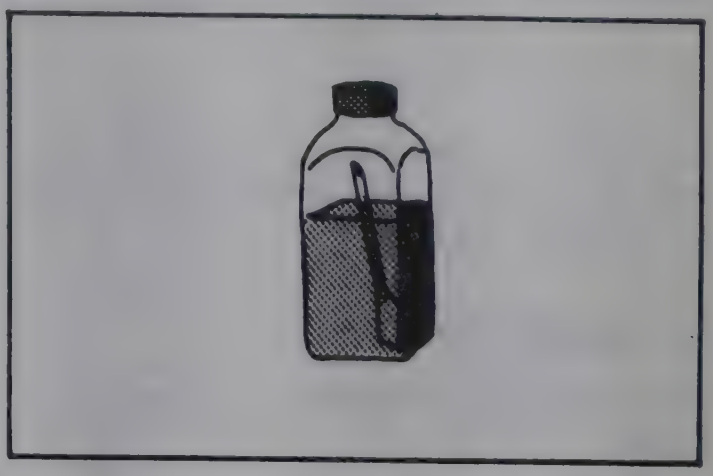
Method:

i. Dip a sterile cotton wool swab in the stool specimen.



ii. For infants or other patients without a stool specimen, take a rectal swab. Moisten the swab with sodium chloride solution (Reagent No. 25) and introduce the swab into the rectum. Turn the swab several times with a circular movement.

iii. Place the swab in a bottle containing Cary-Blair medium ($^3/_4$ full).



If a delay is unavoidable, store at room temperature.

Note: Never store in an incubator.
Never store in a refrigerator.

(2) Blotting paper method

If transport medium is not available:

i. Place a strip of dry blotting paper in a plastic packet.

ii. Pour the watery stool on to the blotting paper strip so that the strip becomes soaked.

iii. Seal the plastic packet to avoid the blotting paper from drying during transit.

CHAPTER 9

Examination of Blood

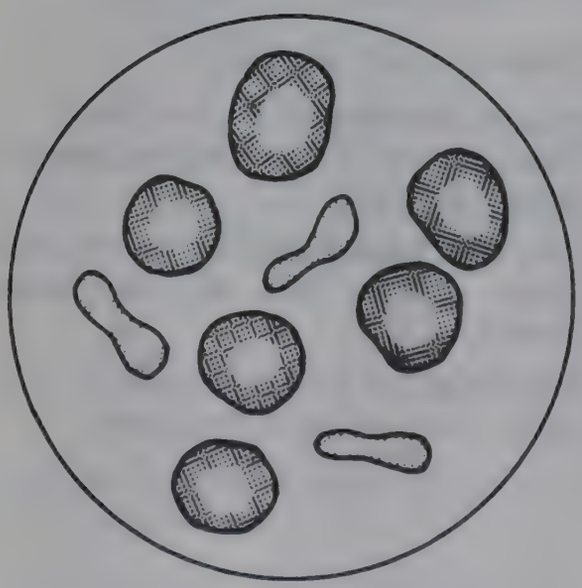
Haematology is the study of the blood, which includes the blood cells and the fluid (plasma) surrounding them.

1. The Blood Cells

Blood cells can be examined under the microscope.

There are 3 different types:

(1) Red blood cells (RBC), also called erythrocytes



Appearance : round cells filled with haemoglobin; red cells on their side look like biconcave discs; they do not contain nuclei
Size : 7.5 μ
Number concentration: about 5×10^{12} per litre, i.e. 5,000,000 per mm^3 of blood
Function : The red cells carry haemoglobin which combines with and carries oxygen from the lungs to the tissues. They also carry carbon dioxide from the tissues to the lungs.

(2) White blood cells (WBC), also called leucocytes

Appearance : round, each containing one nucleus and a few granules
Size : 9–20 μ
Number concentration: about 8×10^9 per litre, i.e. 8,000 per mm^3 of blood
Function : defence of the body against infection.

(3) Platelets, also called thrombocytes

Appearance : fragments of cells of various shapes (triangular, star-shaped, oval, etc), with granules

Size : 2–5 μ
Number
concentration: about 300 × 10^9 per litre, i.e. 300,000 per mm^3 of blood
Function : important in the clotting of the blood.

2. Collection of Capillary Blood by Finger Prick Technique

A few drops of blood taken from the finger (or toe in infants) are enough for certain laboratory examinations, e.g.

- haemoglobin estimation
- total RBC count
- total WBC count
- differential WBC count
- detection of parasites.

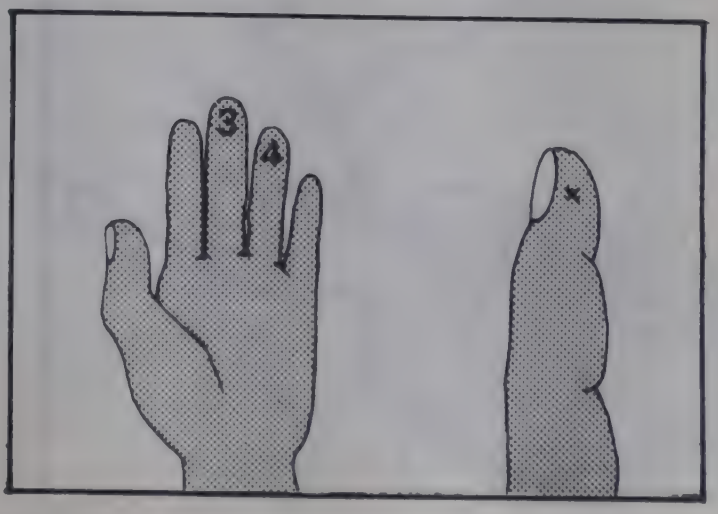
The following may be used for pricking:

- sterile lancet
- Hagedorn needle

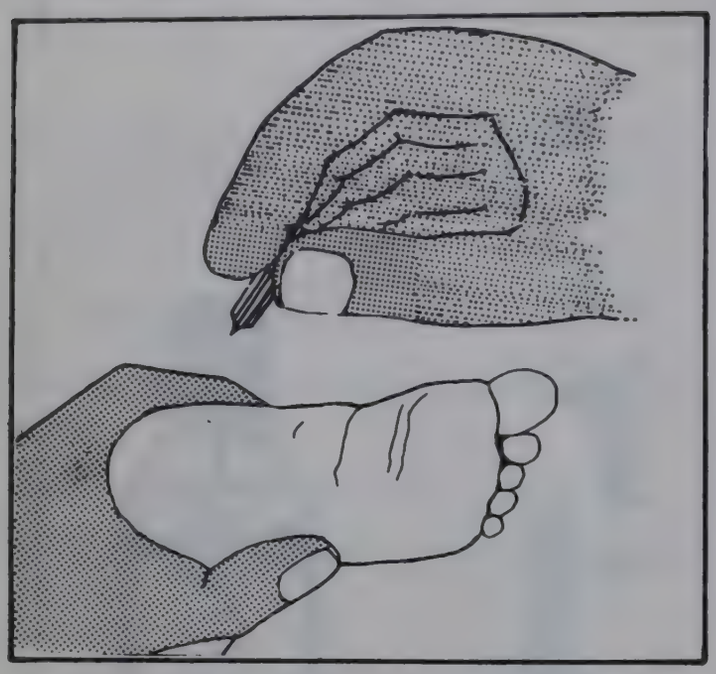
After use the lancets or needles are cleaned and placed in small glass tubes plugged with non-absorbent cotton wool. They are sterilized in the autoclave.

Method

(1) Select the site:
- on the 3rd or 4th finger of the left hand
- at the side of the finger, which is less sensitive than the tip, as shown.

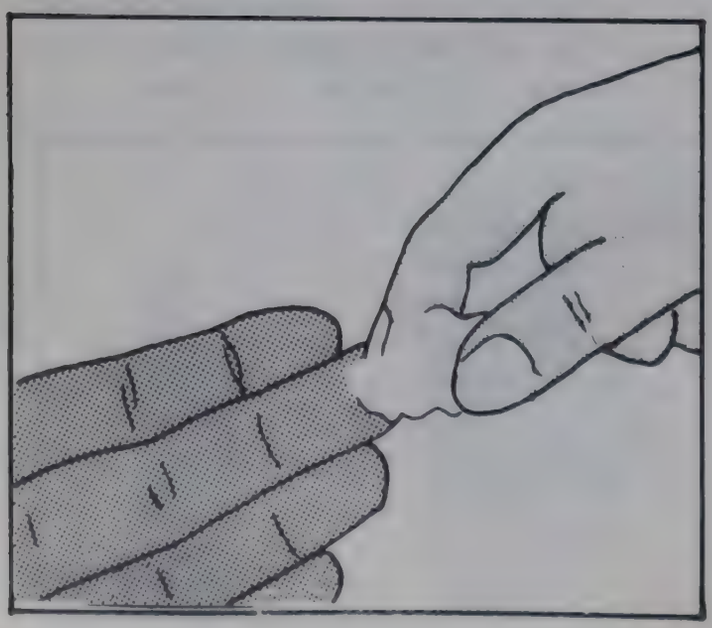


In babies under 6 months:
– prick the heel or big toe.

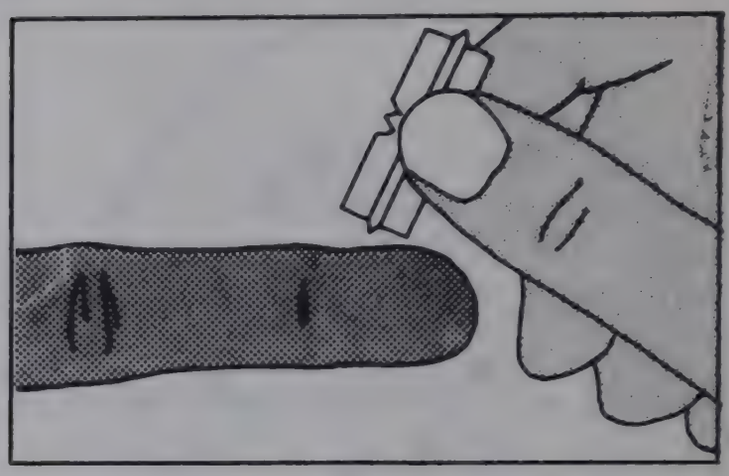


Note: Do not take blood from:
– the index finger or thumb
– an infected finger (paronychia, etc)
– the ear (too many monocytes)

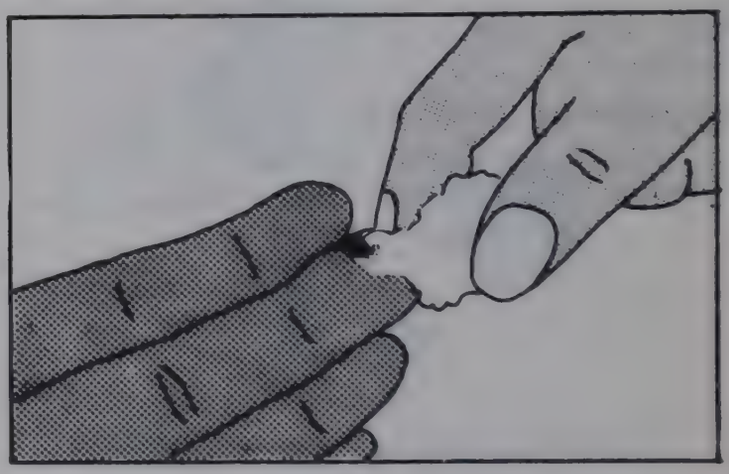
(2) Clean the site:
– first with a cotton wool swab dipped in ethanol
– then with a second dry cotton wool swab or pledget, to remove any ethanol remaining.



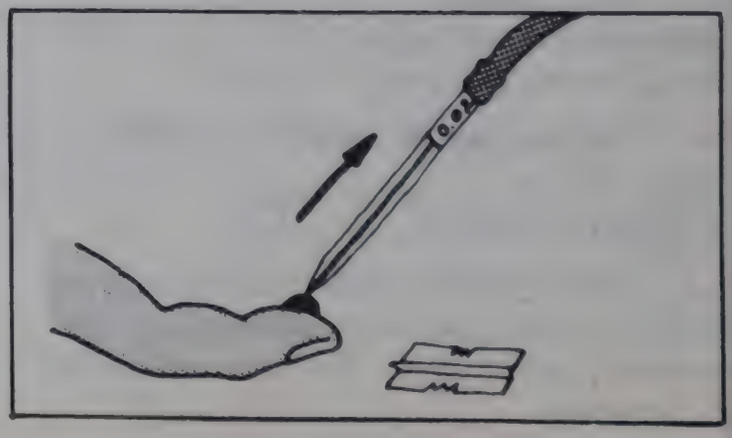
(3) Prick the finger firmly and rapidly.

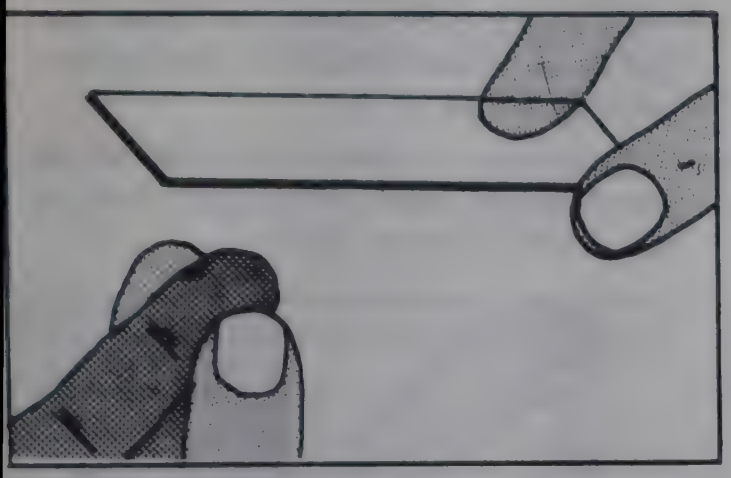


(4) Wipe away the first drop of blood with dry cotton wool.



(5) With your left hand press the finger to produce a drop of blood about this size:
Collect the blood in a pipette or on a slide according to the test which is to be carried out.





If more blood is required, e.g. for measuring the Erythrocyte Sedimentation Rate, the Medical Officer will do a venepuncture and collect the required amount of blood in a bottle containing anticoagulant, e.g. EDTA dipotassium salt solution (Reagent No. 11).

3. Haemoglobin Estimation by Sahli Method

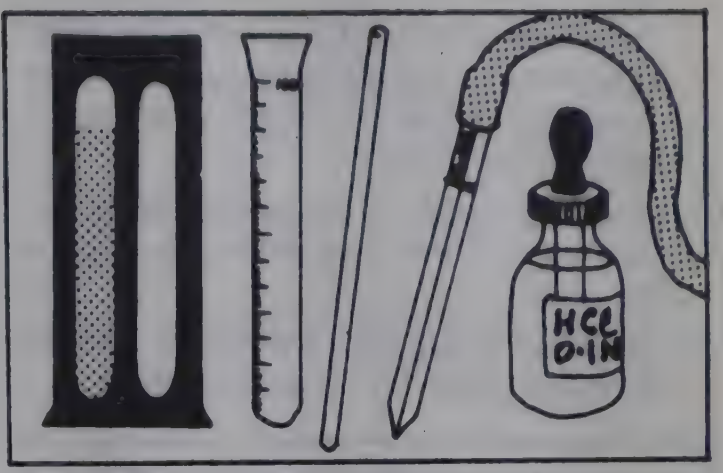
Principle

The blood is diluted in an acid solution, converting the haemoglobin to acid haematin. The test solution is matched against a coloured glass reference.

Note: The Sahli method is not an accurate way of estimating haemoglobin. Not all the forms of circulating haemoglobin are changed into acid haematin; the colour changes, when viewed visually, are not very great; and the brown colour of the standard is not a true match for an acid haematin solution. However, the method is simple and the materials required are available at the PHC.

Materials

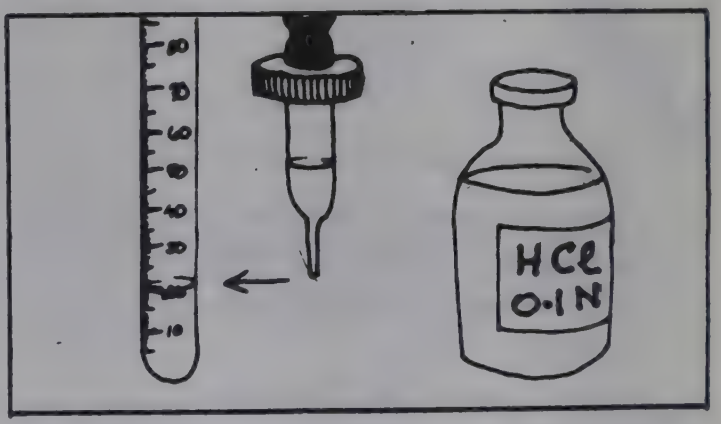
+ Sahli haemoglobinometer
– Sahli pipette graduated to 0.02 ml (20 mm^3 or 20 µl)
– Small glass rod
– Dropping pipette
– Absorbent paper (filter paper)



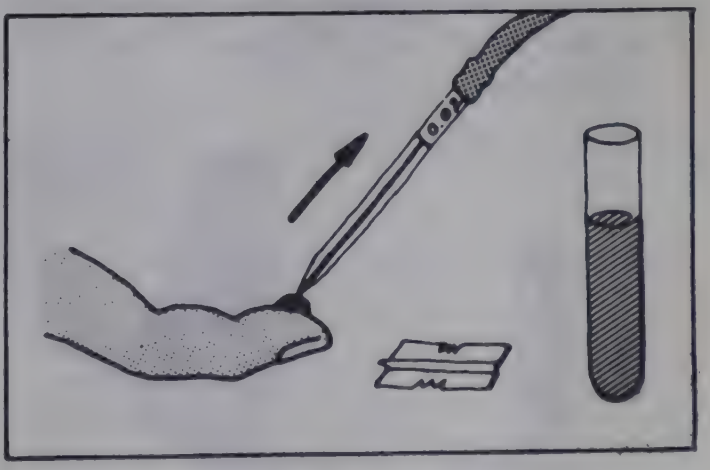
– 0.1 N hydrochloric acid (HCl) (Reagent No. 16.)

Method

(1) Fill the graduated tube to the 20 mark (or the mark 3 g/100 ml) with 0.1 N HCl.



(2) Draw capillary (or venous) blood to the 0.02 ml mark of the Sahli pipette. Do not allow air bubbles to enter.

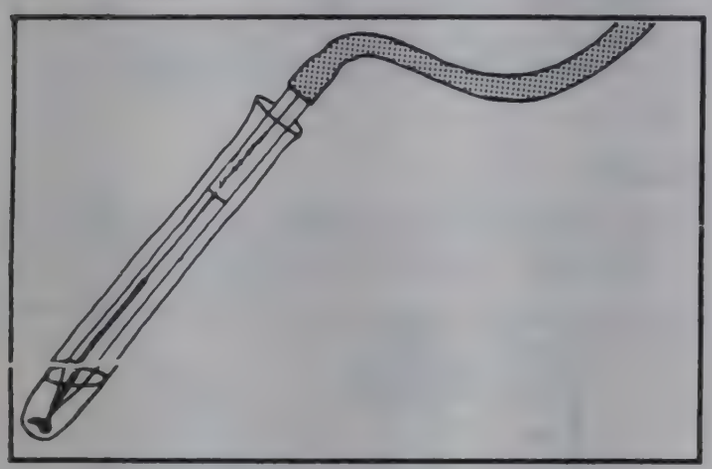


Note: Do not take the first drop of blood from the finger. With venous blood, ensure

that it is well mixed by inverting the bottle containing it and the anticoagulant repeatedly for about 1 minute immediately before pipetting it.

(3) Wipe the outside of the pipette with absorbent paper. Check that the blood is still on the mark.

(4) Blow the blood from the pipette into the graduated tube of the acid solution.



Rinse the pipette by drawing in and blowing out the acid solution 3 times.

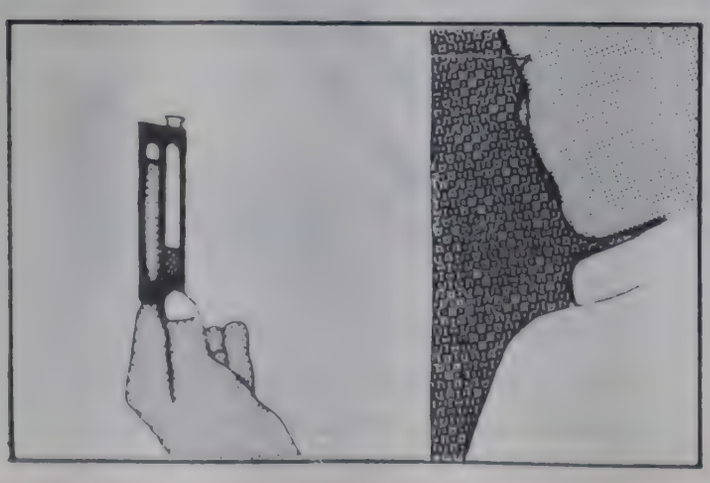
The mixture of blood and acid gives a brownish colour.

Allow to stand for 5 minutes.

(5) Place the graduated tube in the haemoglobinometer.

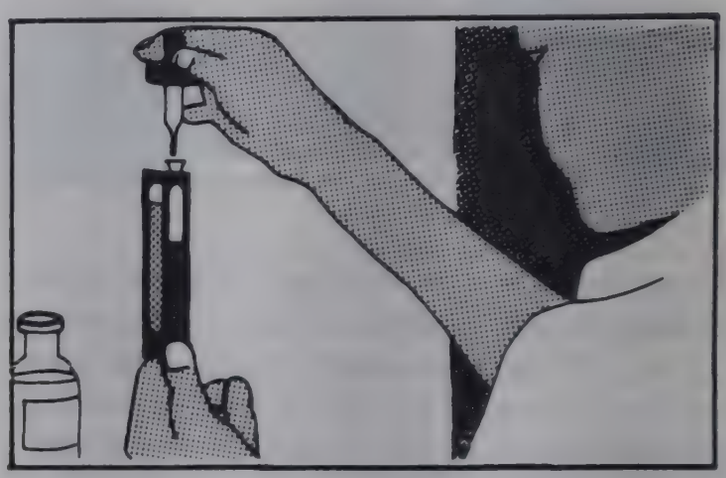
Stand facing a window.

Compare the colour of the tube containing diluted blood with the colour of the reference tube.



If the colour is the same as or lighter than that of the reference tube the haemoglobin value is 40 g/l or less.

(6) If the colour is darker than that of the reference tube, continue to dilute by adding 0.1 N HCl drop by drop.

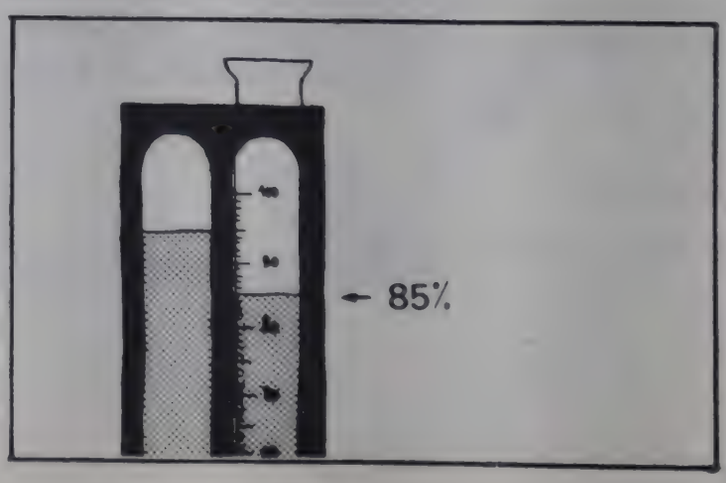


Stir with the glass rod after adding each drop.

Remove the rod and compare the colours of the 2 tubes.

Stop when the colours match. Distilled water can also be used at this step instead of 0.1 N HCl to continue the dilution of the blood.

(7) Note the mark reached. Depending on the type of haemoglobinometer, this gives the haemoglobin concentration either in g/100 ml or as a percentage of 'normal'. (The latter type, which is illustrated, is less accurate.)



To convert g/100 ml to g/l, multiply by 10.

To convert percentages to g/l, multiply by 1.46.

Examples:

(a) 14.8 g/100 ml × 10 = 148 g/l

(b) 85% × 1.46 = 124 g/l

4. Total Erythrocyte (RBC) Count

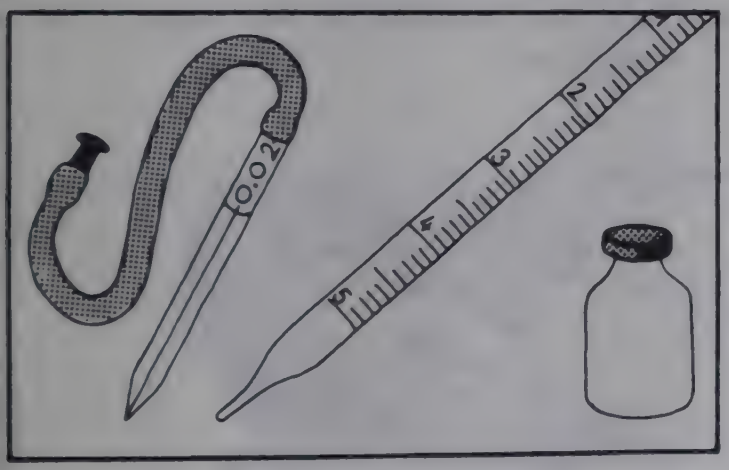
The number of erythrocytes (red blood cells) contained in 1 litre of blood is called the total erythrocyte or red blood cell count. It is usually expressed as the number of cells per cubic millimetre (µl).

Principle

The blood is diluted in a red blood cell diluting fluid.

The RBC are counted in a counting chamber under the microscope and the number of cells per mm^3 of blood is calculated.

Materials

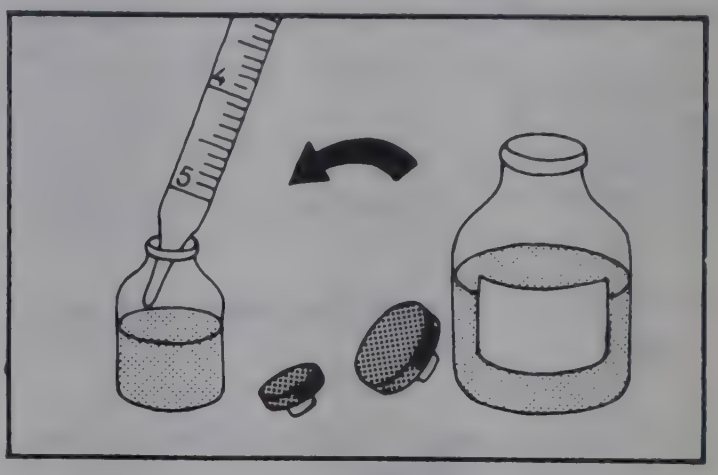


- Blood pipette (often called Sahli pipette) graduated to the 0.02 ml (20 mm^3 or 20 µl) mark, with rubber tubing and mouthpiece

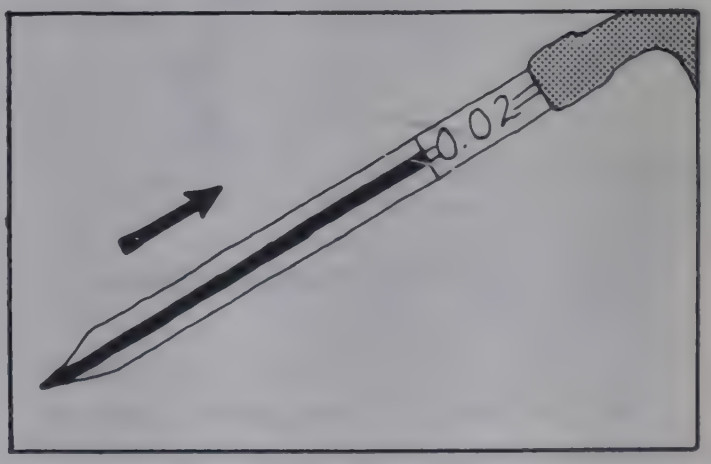
  Note: The use of bulb pipettes is not recommended as they are inaccurate, difficult to use and clean, and more expensive.
- 5 ml graduated pipette
- Pasteur pipette
- Absorbent paper
- Improved Neubauer ruled counting chamber with special coverglass
- RBC diluting fluid (Reagent No. 23)
- Small bottle (an empty clean penicillin vial can be used)
- Paper and pencil

Method

(1) Pipette 4.0 ml of diluting fluid into a small bottle, using the 5 ml graduated pipette.

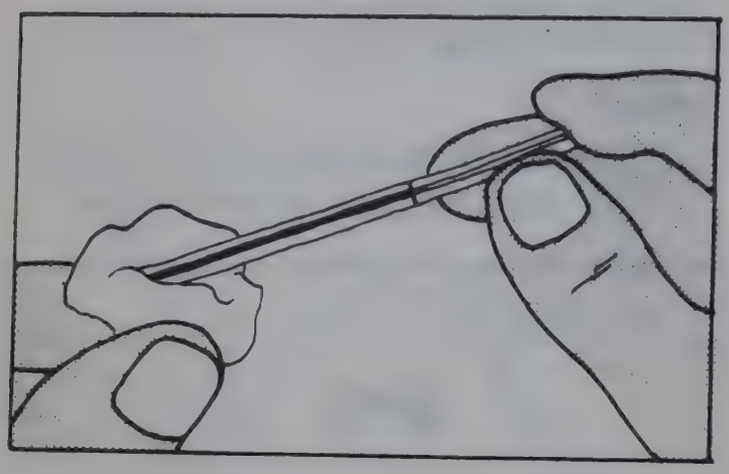


(2) Draw capillary (or venous) blood to the 0.02 ml mark of the blood pipette. Do not allow air bubbles to enter.

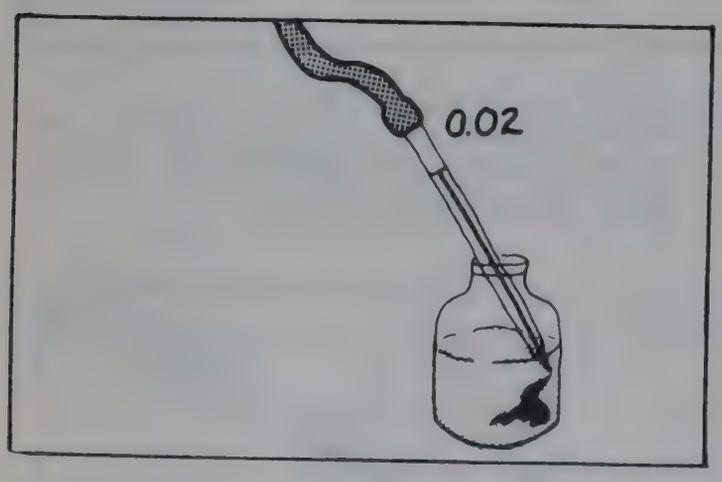


Note: With venous blood, ensure that it is well mixed by inverting the bottle containing it and the anticoagulant repeatedly for about 1 minute immediately before pipetting it.

(3) Wipe the outside of the pipette with absorbent paper. Check that the blood is still on the mark.



(4) Blow the blood into the bottle of diluting fluid.



Rinse the pipette by drawing in and blowing out the fluid 3 times.

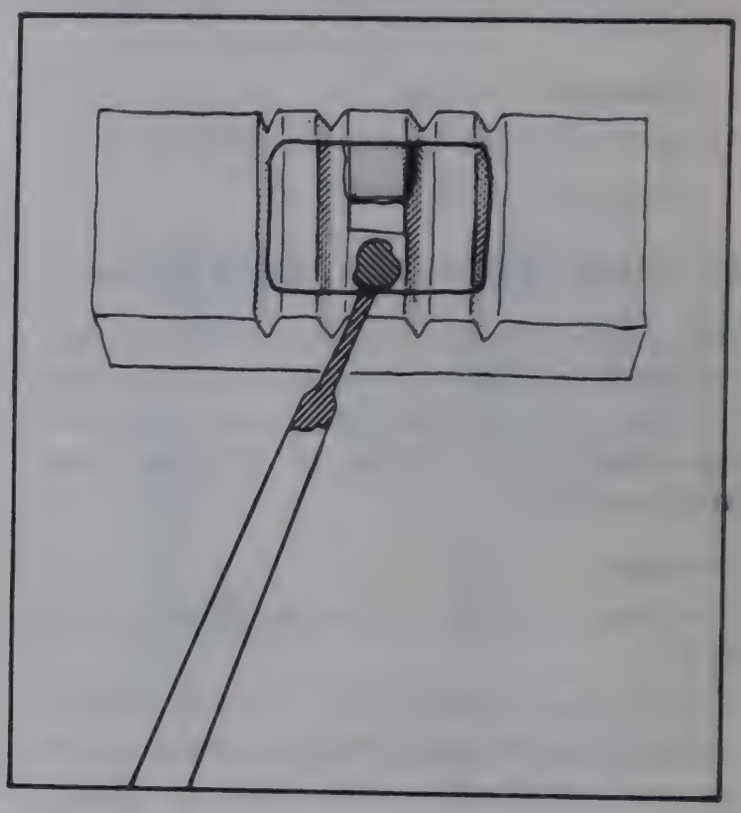
The dilution of the blood is 1 in 200.

Label the bottle with the patient's name or number.

(5) Attach the coverglass to the counting chamber, pressing it carefully into place.

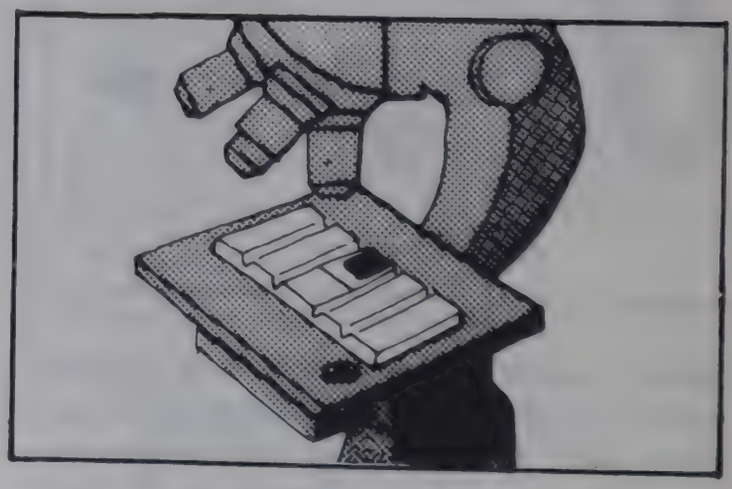
(6) Mix well the diluted blood.

Using a Pasteur pipette, fill the two ruled areas of the chamber. Take care not to overfill beyond the ruled areas.



(7) Leave the counting chamber on the bench for 3 minutes to allow the cells to settle.

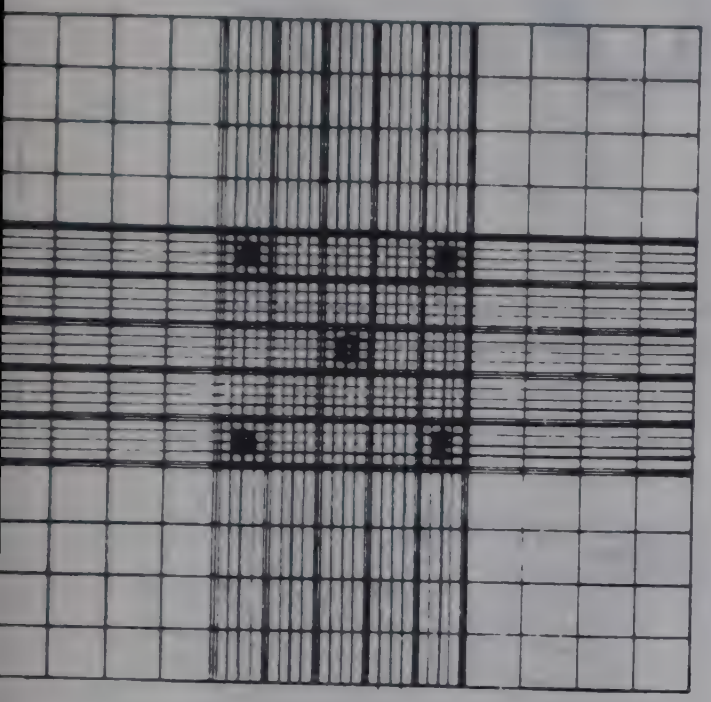
(8) Place the chamber on the stage of the microscope.



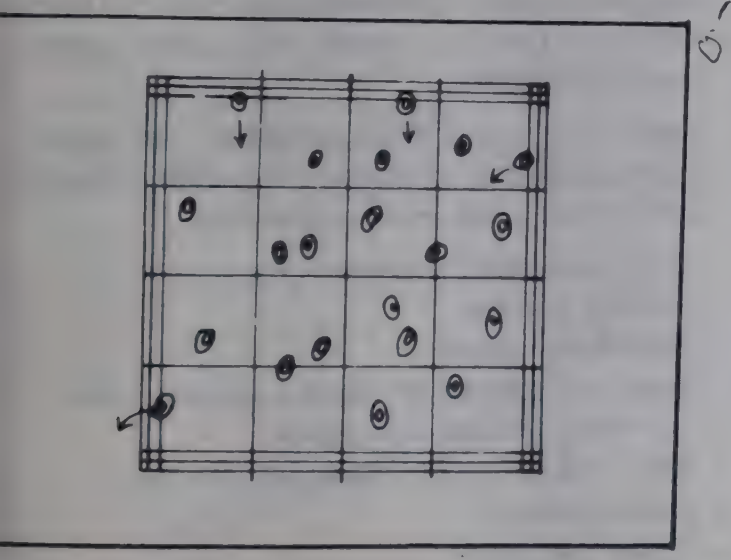
Use the ×10 objective, find the centre square of the chamber, then change to the ×40 objective to count the red blood cells.

Counting of red blood cells (using the improved Neubauer ruled counting chamber)

Count the cells in an area of 0.2 mm^2, using the squares marked A, B, C, D and E.



nclude in the count the cells on the lines of two ides of each square counted as shown in the gure. This square represents one of the five ounted, i.e. A, B, C, D and E.



epeat the count in the second ruled area of the amber.

Calculate the number of cells in 1 cubic millimetre of blood:

- Multiply the number of cells counted in the first group of five squares by 10,000.
- Do the same with the number of cells counted in the second set of five squares.
- Take the average of the two figures.
- Report the result as: Number of cells per mm^3.

Example:

Number of cells counted in (a) first ruled chamber = 390

Number of cells counted in (b) second ruled chamber = 370

Total RBC count (a) = 390 × 10,000
= 3,900,000

Total RBC count (b) = 370 × 10,000
= 3,700,000

Result reported
[average of (a) & (b)] = 3,800,000/mm^3
i.e. 3.8 millions/mm^3

Explanation of calculation:

Each of the five squares in which cells are counted has an area of 0.04 mm^2; the total area is therefore 0.2 mm^2.

The chamber depth is 0.1 mm, therefore the volume in which cells are counted is $0.2 \times 0.1 = 0.02$ mm^3.

Thus division by 2 and multiplication by 100 (i.e. multiplication by 50) will give the number of cells per cubic millimetre of diluted blood.

Since the dilution is 1 in 200, multiplication by 200 will give the number of cells in 1 mm^3 of undiluted blood.

Thus, if the number of cells counted is 380,

$$\text{Cells per } mm^3 = \frac{380 \times 100 \times 200}{2}$$

$$= 380 \times 10{,}000$$

or 3.8 millions/mm^3

(Since the concentration of red blood cells is so high, it is easier to write it in millions.)

Normal range

	Millions of RBC per cubic millimetre
Men	4.5–5.5
Women	4.0–5.0
Children (4 years)	4.2–5.2
Infants (1–6 months)	3.8–5.2
Newborn infants	5.0–6.0

Low values:

Patients with anaemia caused by red blood cell loss or red blood cell haemolysis will have low red blood cell counts.

High values:

Patients who are dehydrated or who have polycythaemia will have high red blood cell counts.

5. Total Leucocyte (WBC) Count

The number of leucocytes (white blood cells) contained in 1 litre of blood is called the total leucocyte or white blood cell count. It is usually expressed as the number of cells per cubic millimetre (μl).

Principle

The blood is diluted in a leucocyte diluting fluid which:

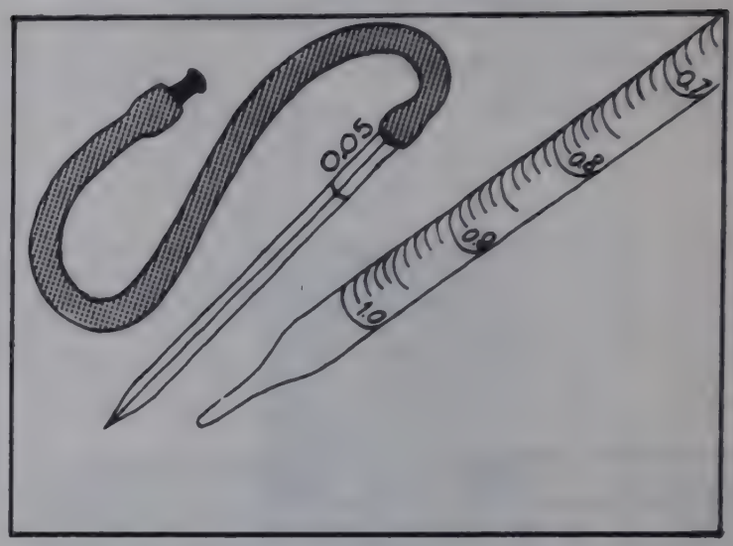
- haemolyses (destroys) the RBC;
- leaves the WBC intact.

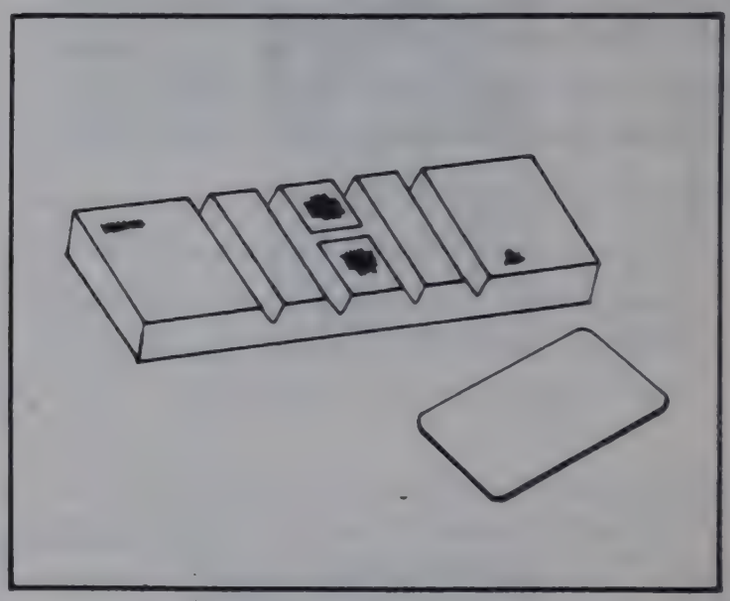
The WBC are then counted in a counting chamber under the microscope, and the number of cells per mm^3 of blood is calculated.

Purpose

In certain diseases the number of leucocytes in the blood is altered. For instance, in some infections there is a marked increase.

Materials



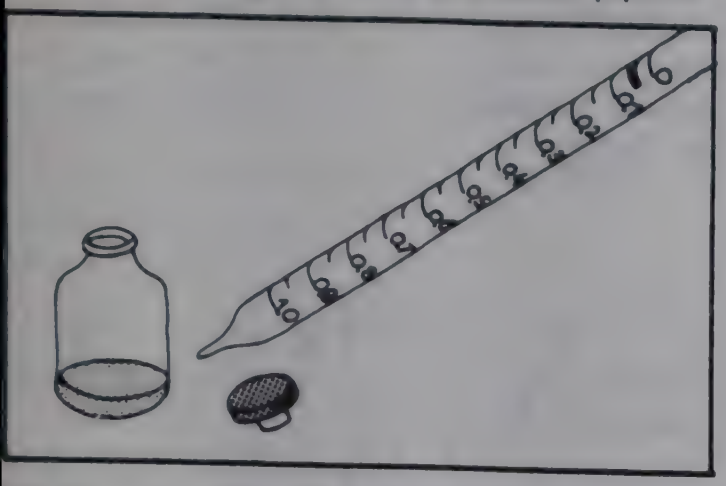


- Blood pipette graduated to the 0.05 ml (50 mm^3 or 50 μl) mark with rubber tubing and mouthpiece

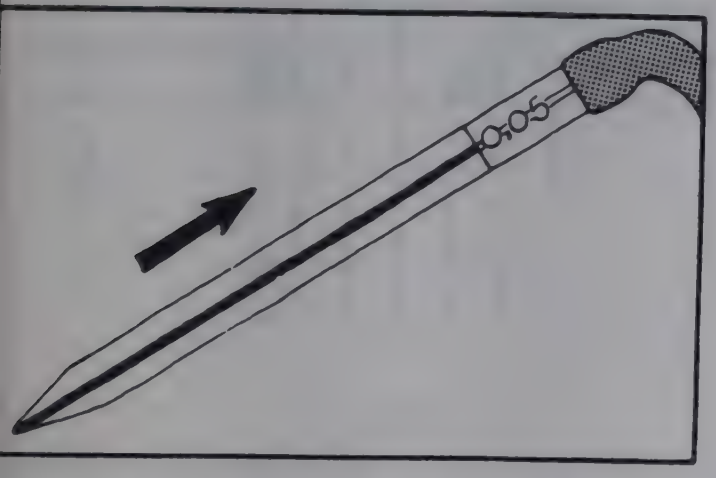
  Note: The use of bulb pipettes is not recommended as they are inaccurate, difficult to use and clean, and more expensive.
- 1 ml graduated pipette
- Pasteur pipette
- Absorbent paper
- Improved Neubauer ruled counting chamber with special coverglass
- WBC diluting fluid (Reagent No.28)
- Small bottle
- Paper and pencil

Method

(1) Pipette 0.95 ml of diluting fluid into a small bottle, using the 1 ml graduated pipette.

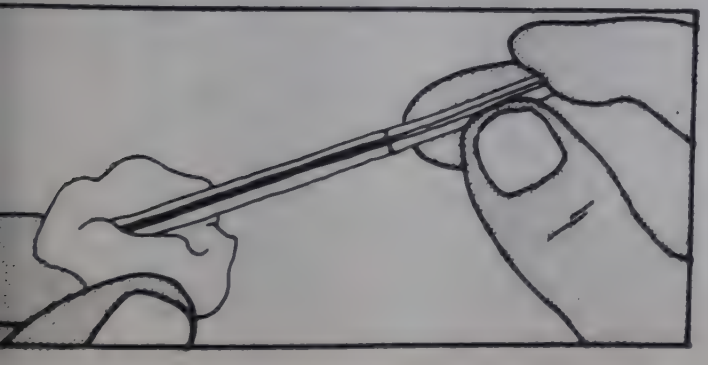


(2) Draw capillary (or venous) blood to the 0.05 ml mark of the blood pipette. Do not allow air bubbles to enter.

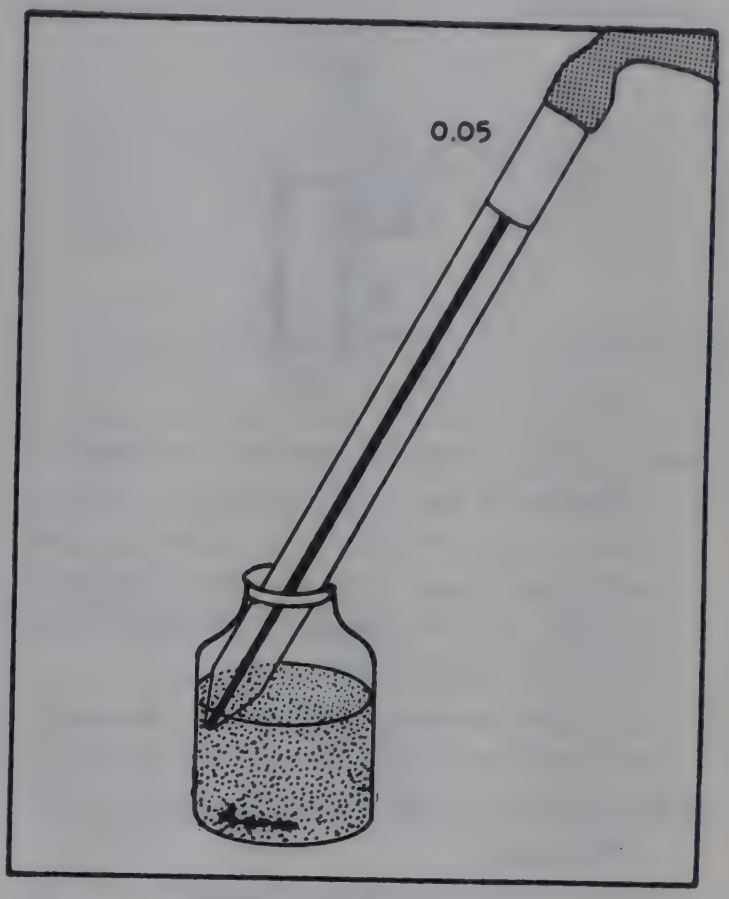


Note: With venous blood ensure that it is well mixed by inverting the bottle containing it and the anticoagulant repeatedly for about 1 minute immediately before pipetting it.

(3) Wipe the outside of the pipette with absorbent paper. Check that the blood is still on the mark.



(4) Blow the blood into the bottle of diluting fluid.

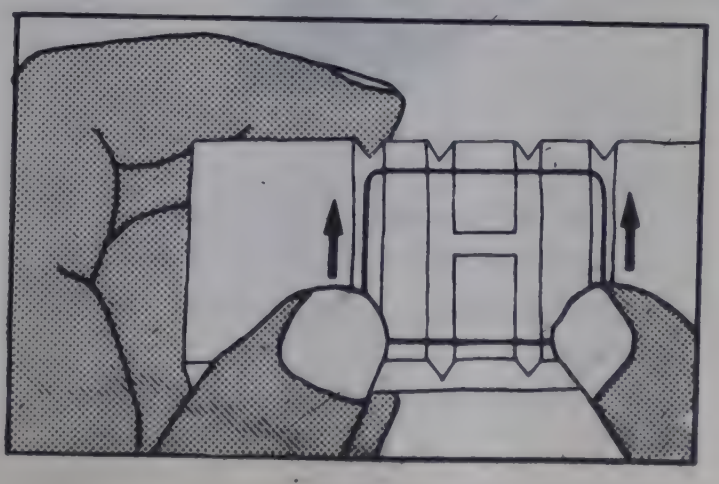


Rinse the pipette by drawing in and blowing out the fluid 3 times.

The dilution of the blood is 1 in 20.

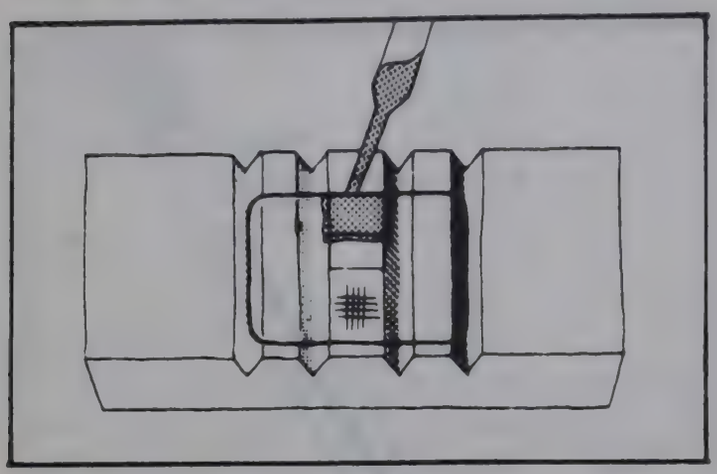
Label the bottle with the patient's name or number.

(5) Attach the coverglass to the counting chamber, pressing it carefully into place.



(6) Mix well the diluted blood.

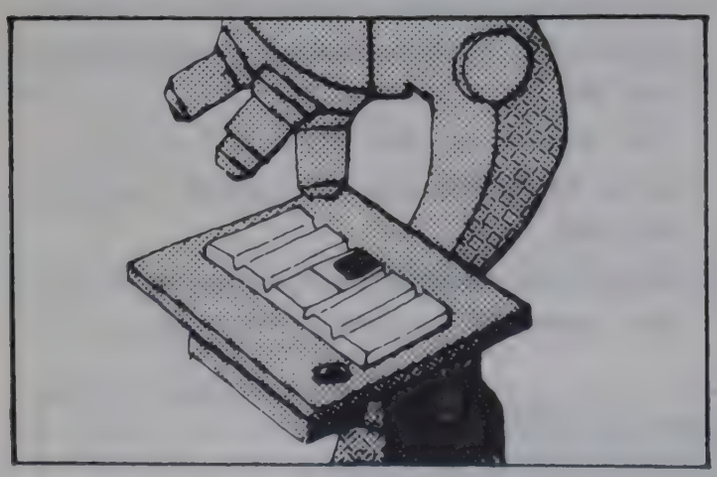
Using a Pasteur pipette fill the counting chamber. Take care not to overfill beyond the ruled area.



Important: If the liquid overflows into the channel between the two chambers, you must start again: remove the coverglass, clean it and the counting chamber, and refill with another drop.

(7) Leave the counting chamber on the bench for 3 minutes to allow the cells to settle.

(8) Place the chamber on the stage of the microscope.



Use the × 10 objective (×5 or ×10 eyepiece).

Reduce the amount of light entering the condenser by adjusting the iris diaphragm.

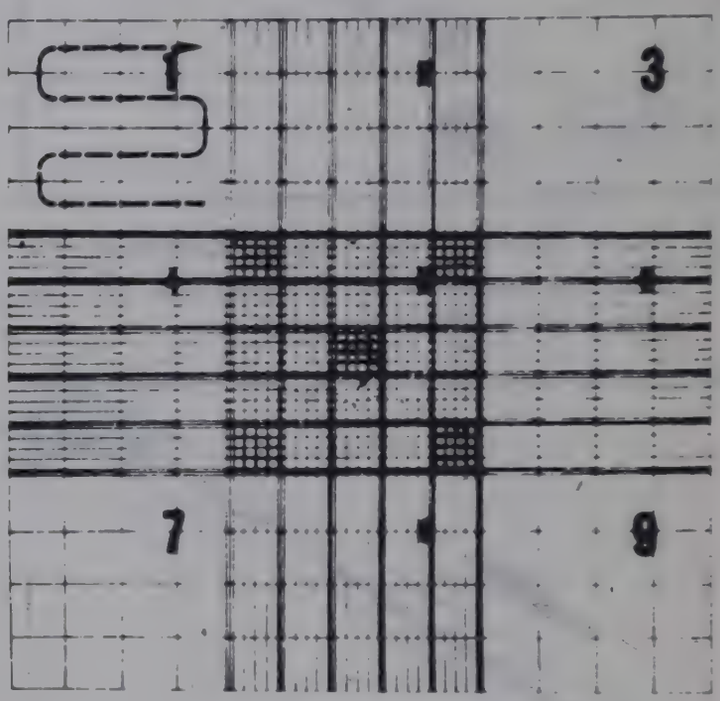
Focus the rulings of the chamber and the leucocytes.

Do not mistake pieces of dust for leucocytes.

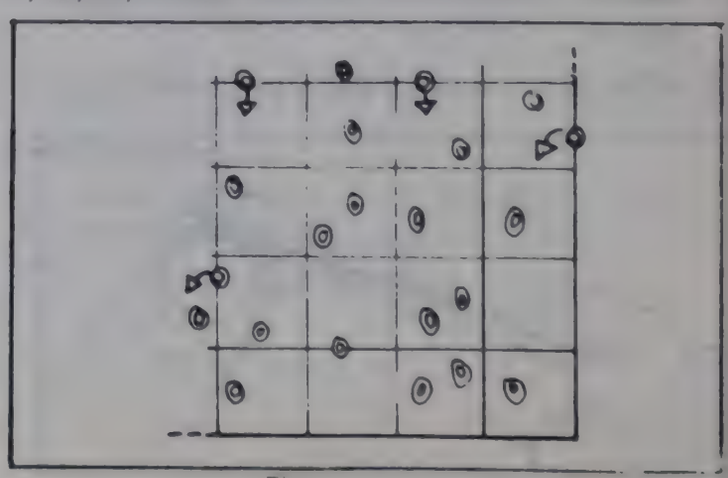
Counting of white blood cells (using the improved Neubauer ruled counting chamber)

- Area of chamber = 9 mm^2
- Depth of chamber = 0.1 mm

Count the cells in an area of 4 mm^2, using the squares numbered 1, 3, 7, and 9 as shown in the diagram.



Include in the count the cells seen on the lines of two sides of each square counted, as shown in the figure. This square represents one of the four counted, i.e.
1, 3, 7, and 9.



Calculate the number of cells in 1 cubic millimetre of blood:

- Multiply the number of cells counted in the four squares by 50.
- Report the result as: Number of cells per mm^3.

Example:

Number of white blood cells counted = 188
Total WBC count = 188 × 50
Result reported: 9,400/mm^3

Explanation of calculation:

Each of the four squares in which cells are counted has an area of 1 mm^2; the total area is therefore 4 mm^2.

The chamber depth is 0.1 mm, therefore the volume in which cells are counted is $4 \times 0.1 = 0.4$ mm^3.

Thus division by 4 and multiplication by 10 will give the number of cells in 1 mm^3 of diluted blood.

Since the dilution is 1 in 20, multiplication by 20 will give the number of cells in 1 mm^3 of undiluted blood.

Thus, if the number of cells counted is 188,

$$\text{cells per } mm^3 = \frac{188 \times 10}{4} \times 20$$

$$= 188 \times 50$$
$$= 9{,}400/mm^3$$

Normal range

	WBC per cubic millimetre
Men and women	4,000–10,000
Children of 10 years	4,000–10,000
Children of 3 years	4,000–11,000
Infants (3–9 months)	4,000–15,000
Newborn infants	10,000–12,000

High values:

An increase in the total number of circulating white blood cells is called leucocytosis. This can occur with certain pyogenic bacterial infections. In leukaemia, the leucocyte count can reach 50,000/mm^3 to 400,000/mm^3 or even higher values.

It is then necessary, when determining the total leucocyte count, to use a greater dilution of blood—for example, 0.05 ml of blood and 1.95 ml of diluting fluid, which gives a dilution of 1 in 40. If this dilution is used, the number of cells counted is multiplied by 100 instead of 50 to give the number per cubic millimetre.

Low values:

A decrease in the total number of circulating white blood cells is called leucopaenia. This can occur with certain infections including typhoid fever and malaria. Leucopaenia is also seen following treatment with certain drugs.

When the total leucocyte count is very low, it is necessary to dilute the blood less—for example, 0.05 ml of blood and 0.45 ml of diluting fluid, which gives a dilution of 1 in 10. If this dilution is used, the number of cells counted is multiplied by 25 instead of 50 to give the number per cubic millimetre.

6. Cleaning Blood Pipettes and Counting Chambers

(1) Cleaning materials and preparations

A wide range of cleaning materials should be kept for the maintenance of all the equipment—pipettes, counting chamber, coverglass, etc. The items recommended here are routine and easy to obtain:

- Rubber bulb
- Fine nylon thread
- 500 ml and 50 ml beakers
- 95% ethanol. Ether. Acetone
- 0.5% acetic acid in water:

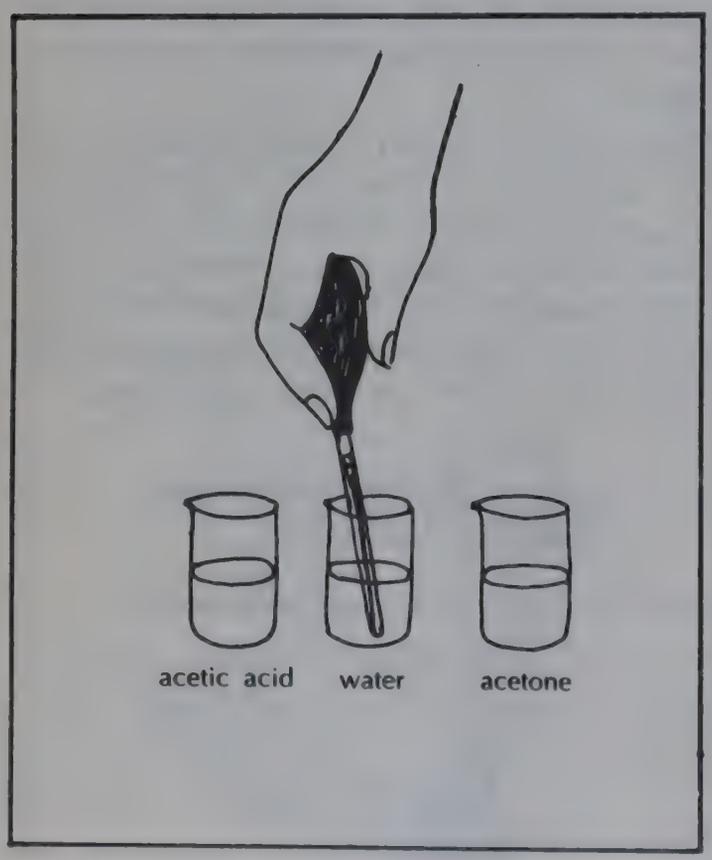
acetic acid	5 ml
distilled water	1000 ml

– Dichromate cleaning solution (Reagent No. 10)
– Sodium bicarbonate (if available)

(2) Pipettes: daily cleaning

i. Soaking: soak pipettes in clean tap water.

ii. Cleaning: using a rubber bulb, draw through each pipette the following:
– dilute acetic acid (3 times)
– clean water, distilled if plentiful (4 times)
– air (hold the pipette tip upwards)
– acetone (once)
– air (to dry as much as possible).



iii. Drying: keep pipettes upright in a container padded with non-absorbent cotton wool.

Leave overnight, if possible at 37°C.

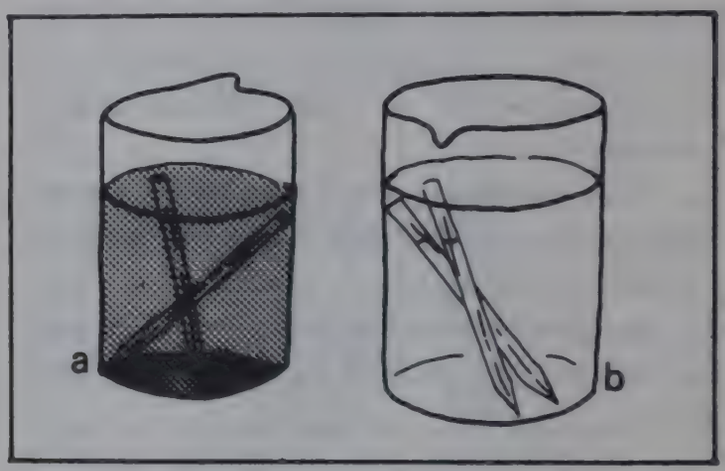
If the pipette is required at once use:
– water, preferably distilled
– ether
– dry completely in the air.

(3) Pipettes: monthly cleaning (more often if necessary)

i. Soak the pipettes in a beaker of dichromate cleaning solution for about 12 hours (a).

ii. Rinse the outside, then the inside, with ordinary tap water.

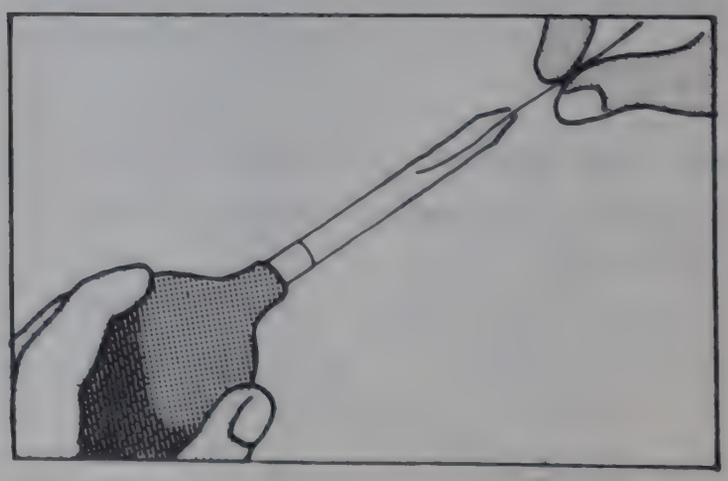
iii. Soak the pipettes in a beaker of weak sodium bicarbonate solution (approximately 10 g/l; 1%) for 12 hours (b).



iv. Clean as described above under 'daily cleaning'.

(4) Blocked pipettes

Try to force a fine nylon thread (000 type) through the mouth of the pipette while applying suction at the other end.



If this fails, leave to soak for 12 hours in a beaker of dichromate solution.

(5) Cleaning of counting chamber and coverglass

Clean as soon as possible after use:
- soak in a solution of detergent powder for 2 to 3 hours
- rinse well under running tap water
- rinse with ethanol, if available
- dry with a soft, non-fluffy cloth.

Never use scouring powder as it will scratch the ruled surface of the chamber.

When several tests are done, clean as soon as possible after use by rinsing in water and wiping with a soft clean cloth. Once a month the chamber needs a more thorough cleaning, as above.

7. Differential Leucocyte (WBC) Count

The leucocytes (white blood cells) in the blood are not all identical. There are five main types of leucocytes, which differ in size, shape of the nucleus, colour of the granules in the cytoplasm, and other factors. The proportion of each leucocyte type is of diagnostic importance. The proportion of each type is called the differential leucocyte count.

Principle

A thin smear is prepared by spreading a small drop of blood evenly on a slide so that there is only one layer of cells.

100 leucocytes are counted, and the number of each type seen is recorded. The proportion of each leucocyte type is reported as a percentage, e.g.

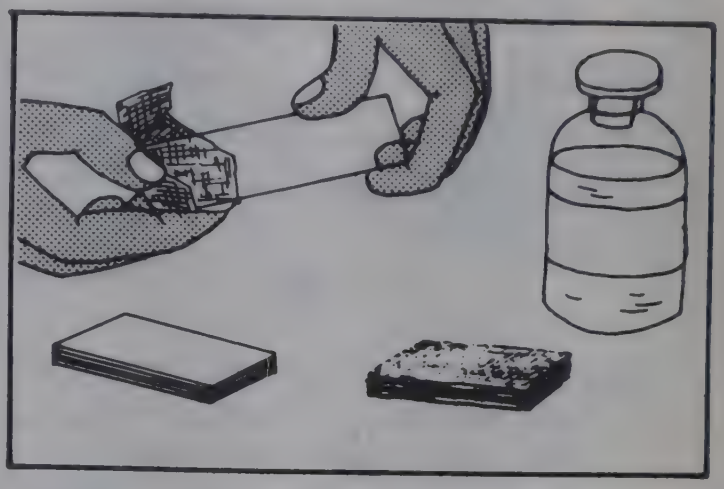
Neutrophils	56%
Lymphocytes	25%
Eosinophils	12%
Monocytes	6%
Basophils	1%

The total of all the percentages should be 100%.

(1) Preparation of thin blood film

Materials

- Clean grease-free glass slides. Slides to be used for thin blood films should be well washed and, if necessary, cleaned with ethanol/ether using a piece of soft cloth.

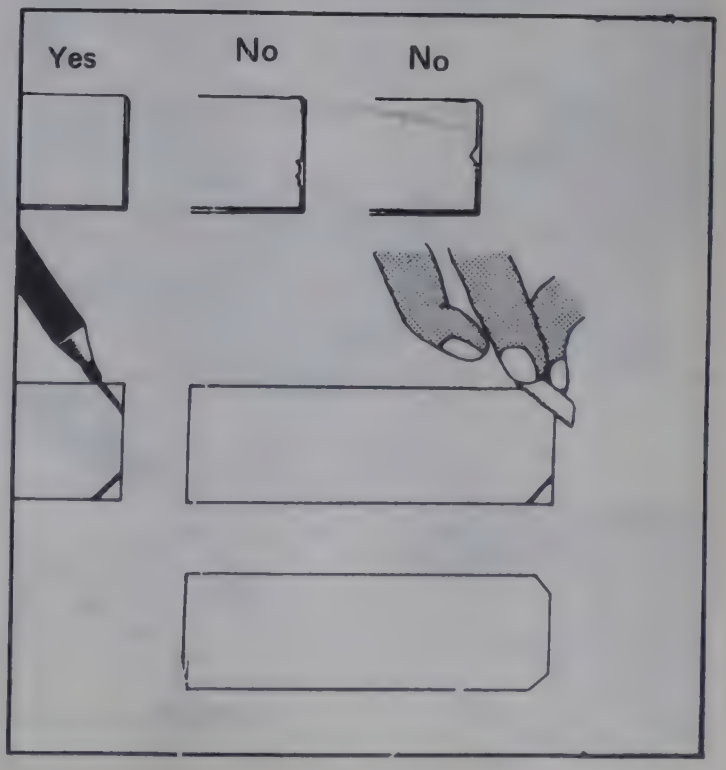


Making a glass spreader:

i. Select a slide with a perfectly smooth edge.

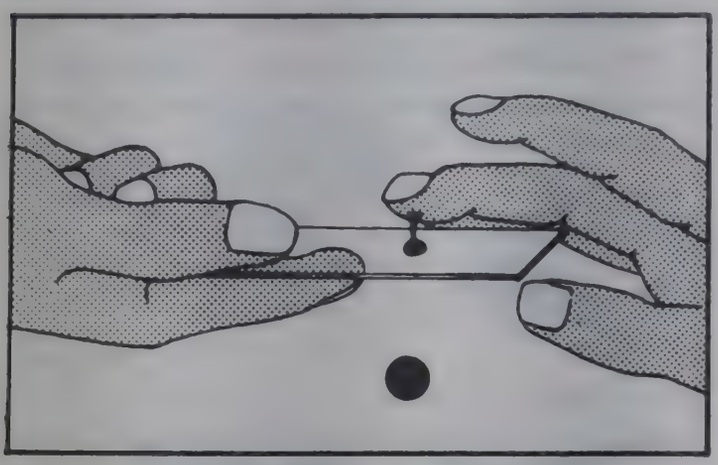
ii. Make a diagonal mark across the two corners at one end of the slide with a file.

iii. Snap off the two filed corners.

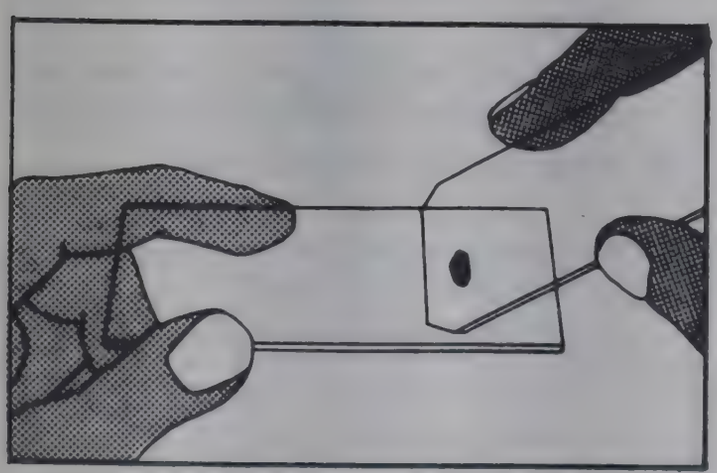


Method

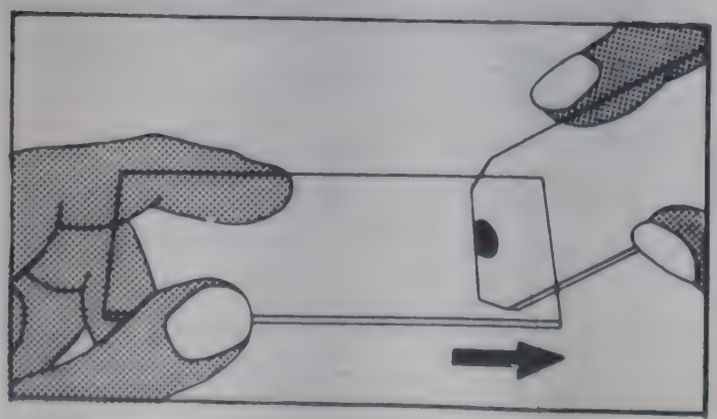
i. Collect a drop of blood of about the size shown by touching it lightly with one end of the slide.



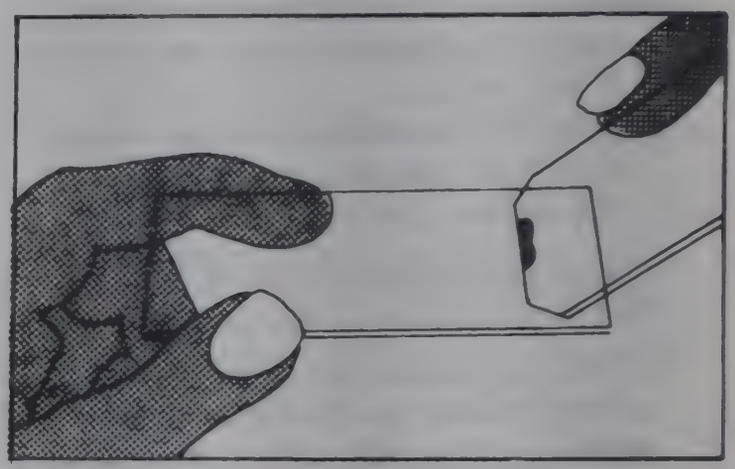
ii. Hold the slide with one hand. Using the other hand, place the edge of the spreader just in front of the drop of blood.



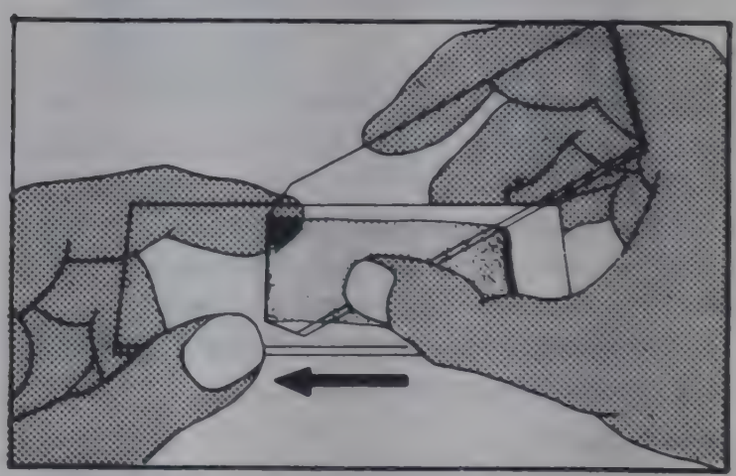
iii. Draw the spreader back until it touches the drop of blood.



iv. Let the blood run along the edge of the spreader.



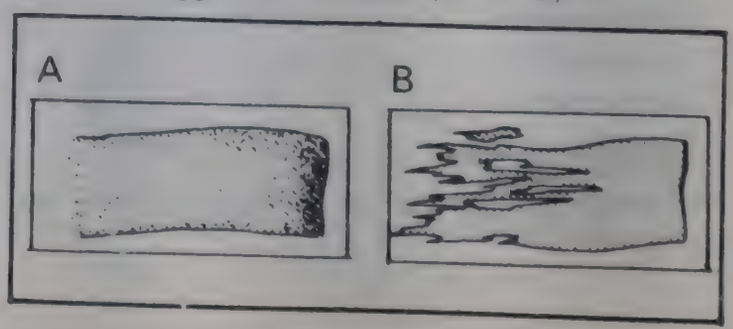
v. Push the spreader to the end of the slide with a smooth movement (all the blood should be used up before you reach the end).



Blood from patients with anaemia should be spread more rapidly.

vi. Check that the film is satisfactory (as in A):

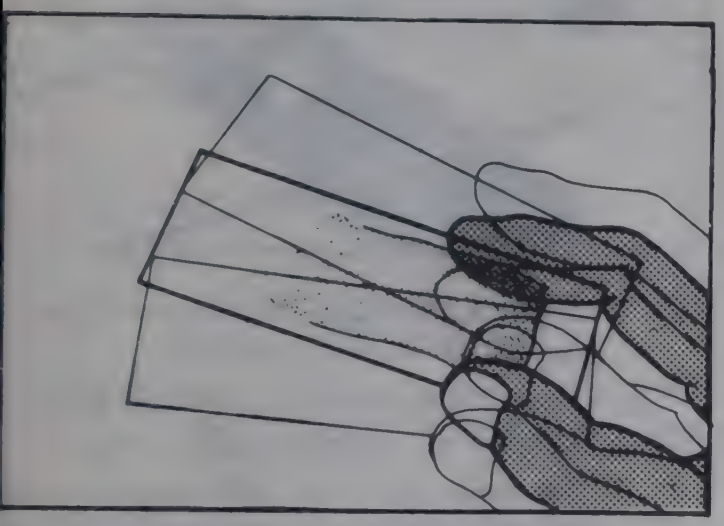
- there should be no lines extending across or down through the film
- the film must be smooth at the end, not ragged and lined (as in B)



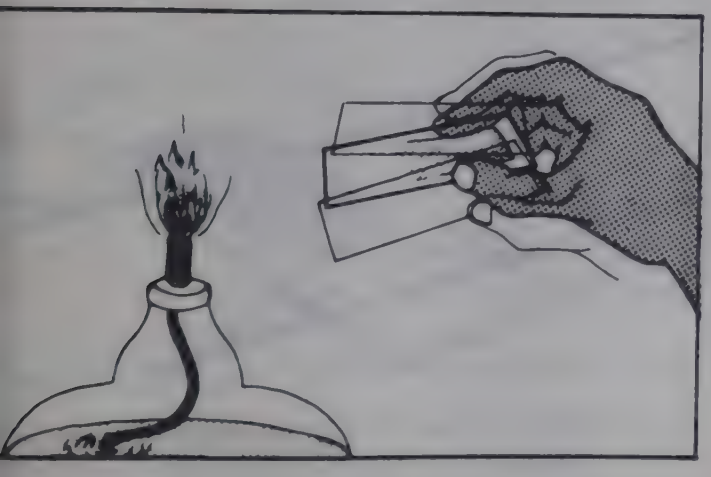
– the film must not be too long
– the film must not be too thick
– the film must not contain holes because a greasy slide has been used.

A well spread film is of great importance. A badly spread film will give a wrong differential leucocyte count.

vii. Adequate drying is essential to preserve the quality of the film, especially in humid climates.

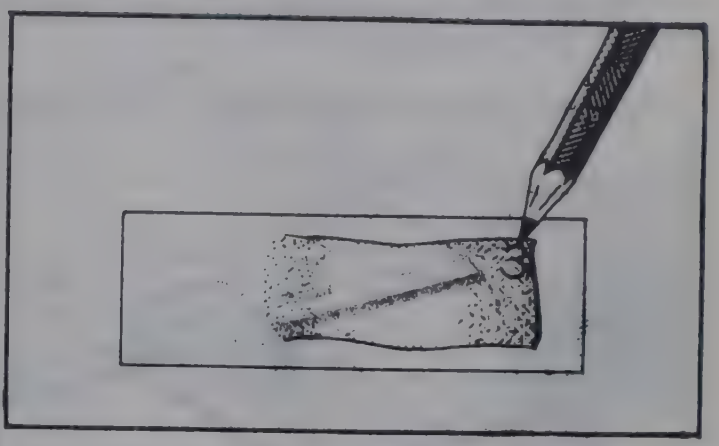


In the wet season: Dry the film by waving it rapidly about 5 cm away from the flame of a spirit lamp; to the side and slightly above (but never directly over) the flame.



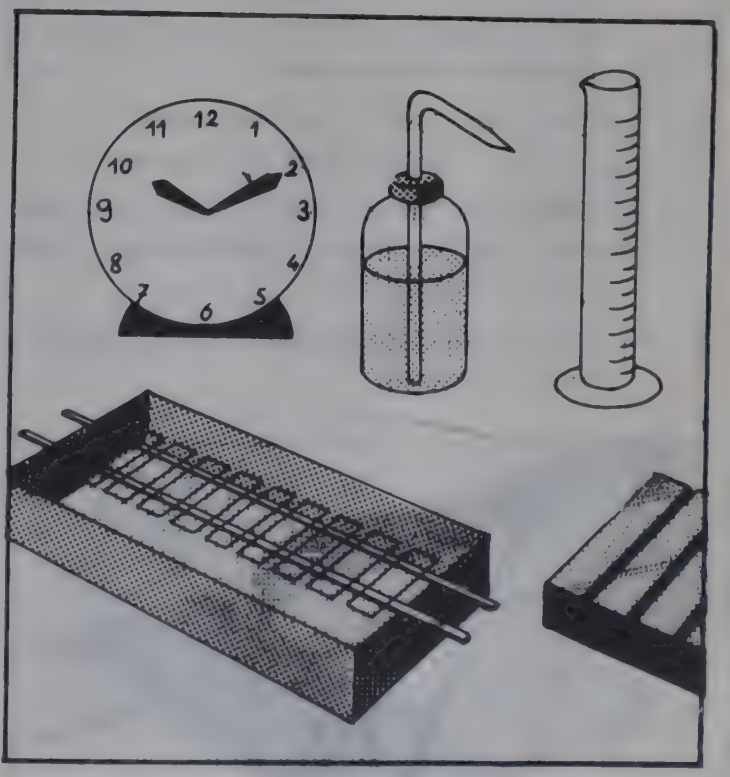
Protect the blood film from flies and dust.

viii. Mark the dry film with the patient's name or number. Write with a lead pencil on the thick part of the film not used for examination.



(2) Staining of thin blood film with Leishman stain

Materials

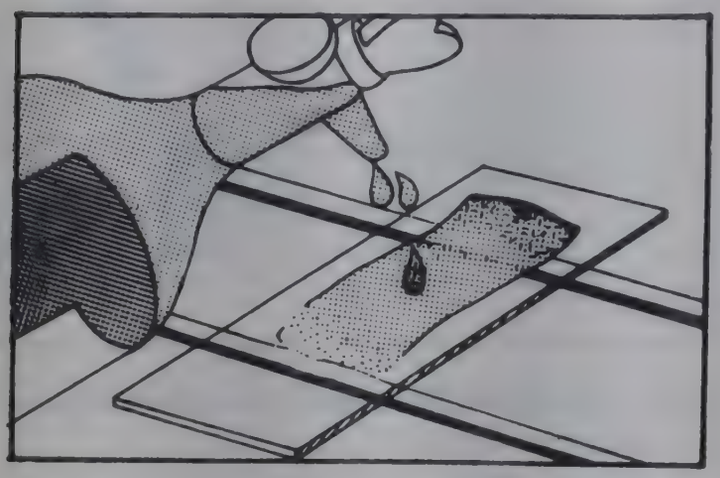


– 2 glass rods either over a sink or over a staining tank
– Measuring cylinder 50 ml or 100 ml
– Wash bottle containing buffered water (Reagent No. 5)
– Interval timer clock
– Rack for drying slides

– Leishman stain (Reagent No. 18)
– Methanol

Method

i. Fix the thin blood film with methanol for 2–3 minutes.

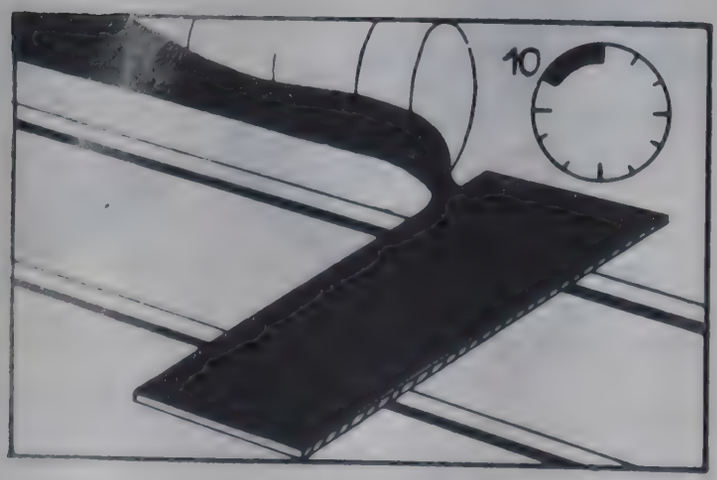


ii. Prepare a 1 in 3 dilution of Leishman stain using 1 part of stain and 2 parts of buffered water. Mix.

Example: Use 10 ml stain and
20 ml buffered water.

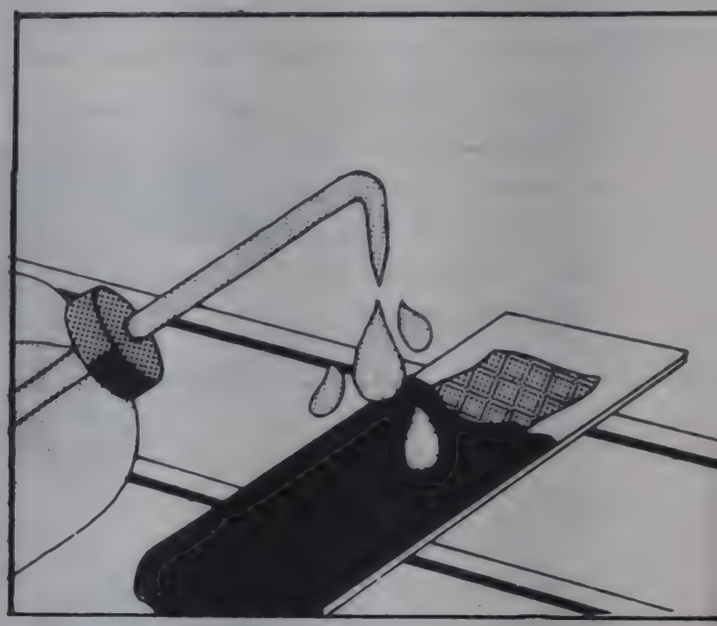
Prepare sufficient stain for one day's use only, as the diluted stain does not keep well.

iii. Cover the slide with the diluted stain for 7–10 minutes.



Note: The staining time may need changing, especially when a new batch of stain is received or the stain has been stored for a long time.

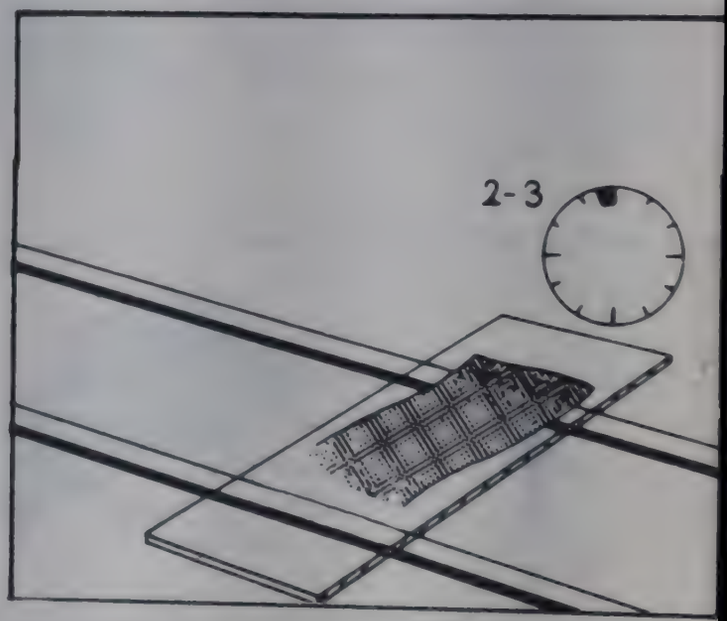
iv. Wash the stain off in a stream of buffered water.



Do not tip the stain off as this will leave a deposit of stain on the film.

v. Leave clean water on the slide for 2–3 minutes to differentiate the film.

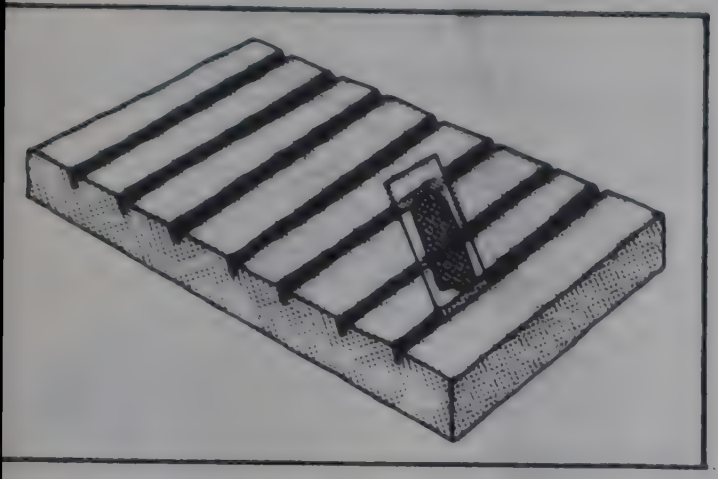
The time taken for differentiation will depend on the stain and the pH of the water used.



The pH is of vital importance in differentiating leucocytes with Leishman stain.

It should be between 6.8 and 7.2, and preferably between 7.0 and 7.2.

vi. Tip the water off and stand the slide in a draining rack to dry.



Results

n a well stained film:

Neutrophils	: The cytoplasm stains faint pink and contains small mauve granules
Eosinophils	: The cytoplasm stains faint pink and contains large red granules
Monocytes	: The cytoplasm stains grey blue
Lymphocytes	: Large – the cytoplasm stains a clear blue Small – the cytoplasm stains a dark blue
Basophils	: Many dark mauve-blue granules fill the cell
Red blood cells	: Stain pink red
Platelets	: Stain mauve-pink

(3) Counting the types of leucocytes

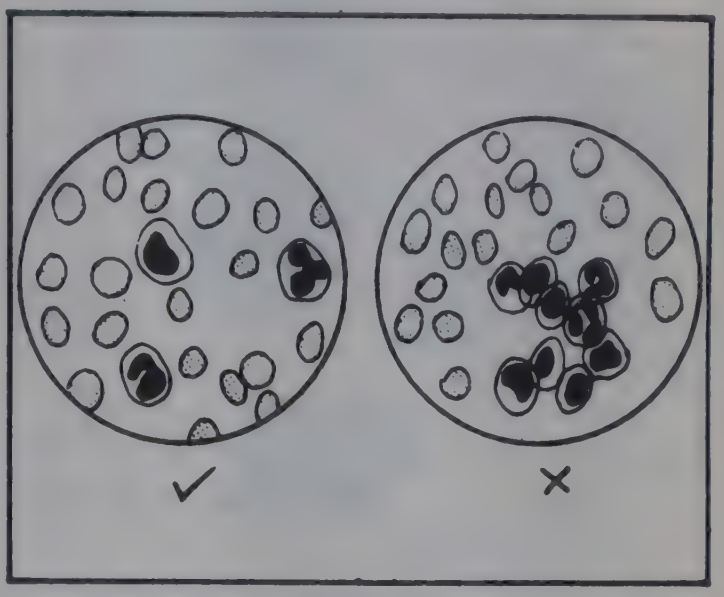
Materials

- Microscope (× 5 eyepiece and × 100 oil immersion objective; a × 40 dry objective with coverslips is also used
- Immersion oil
- Well spread thin blood film stained with Leishman stain
- If available a bead counter
  or paper and pencil

Method

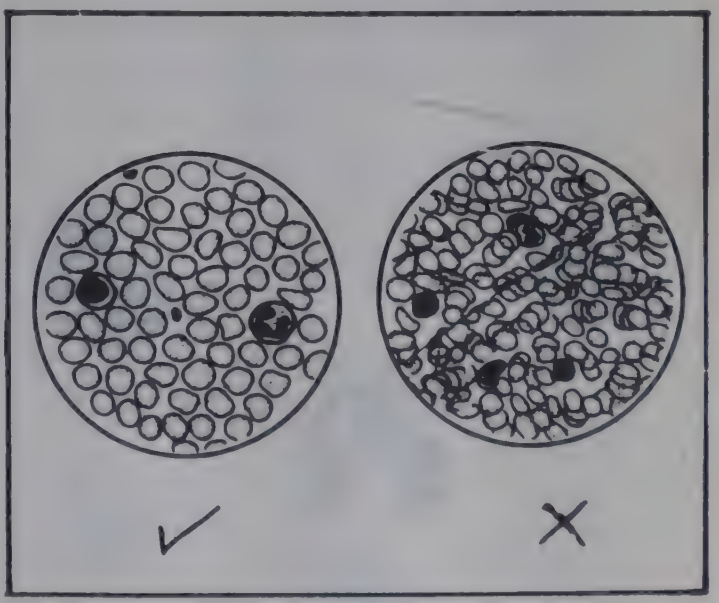
i. Using the × 100 oil immersion objective, check that the white blood cells are evenly distributed.

In a badly spread film the neutrophils may have collected at the end of the film.



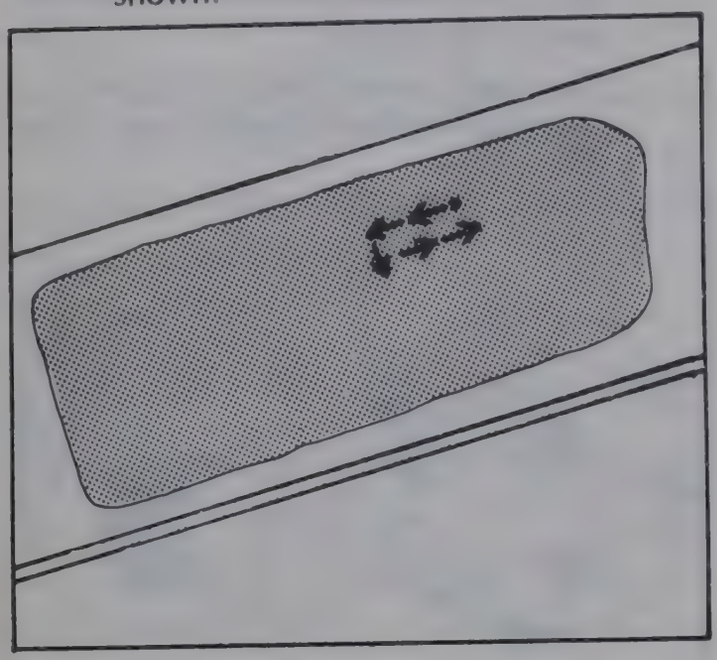
Check that the film is not too thick.

If you see that the film is getting thick (red cells very crowded), stop moving towards the front end; move across and towards the end of the film.



ii. Begin the count near the end of the smear, just where the red cells are beginning to overlap.

iii. Examine a strip of the film, moving from one field to the next systematically, as shown.

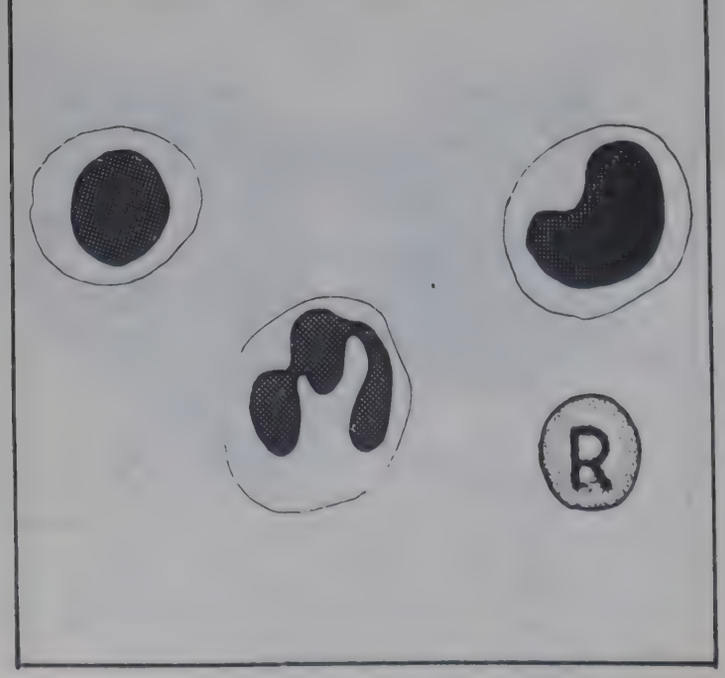


Record the type of leucocytes seen in each field.

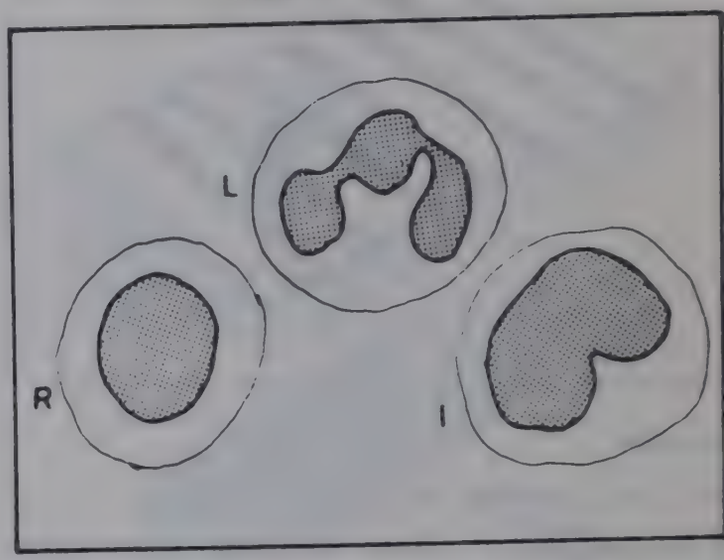
iv. Count a total of 100 leucocytes.

Examination of leucocytes

i. Note the shape of the leucocyte and its size in comparison with that of a red blood cell (R).



ii. Note the shape of the nucleus and its size in relation to the total area of the cell: – round (R)
– lobed (L)
– indented (I)



iii. Note the appearance of the cytoplasm.

Colour:
– colourless
– pink
– pale blue
– dark blue

Granules in the cytoplasm:
– neutrophil granules (N) – mauve, small
– eosinophil granules (E) – orange-red, large
– azurophil granules (A) – bright reddish purple, quite large
– basophil granules (B) – deep purple, very large

olymorphonuclear neutrophil cells (P)

ave: – a nucleus with several lobes
– granules in the cytoplasm (hence their usual name: granulocytes).

mphocytes (L) and Monocytes (M)

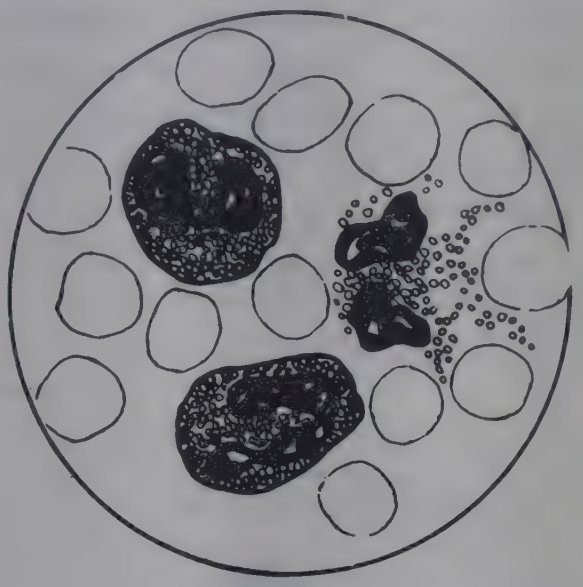
ave: – a compact nucleus
– with or without granules in the cytoplasm.

i. *Polymorphonuclear neutrophil cell*

Size	: 12–15 μ
Shape	: rounded, well defined
Cytoplasm	: abundant, pinkish
Granules	: mauve and very small, numerous but separate
Nucleus	: several (2–5) lobes, linked by strands of chromatin. The chromatin appears as a uniform deep purple mass.

(The older the cell, the greater the number of lobes in the nucleus.)

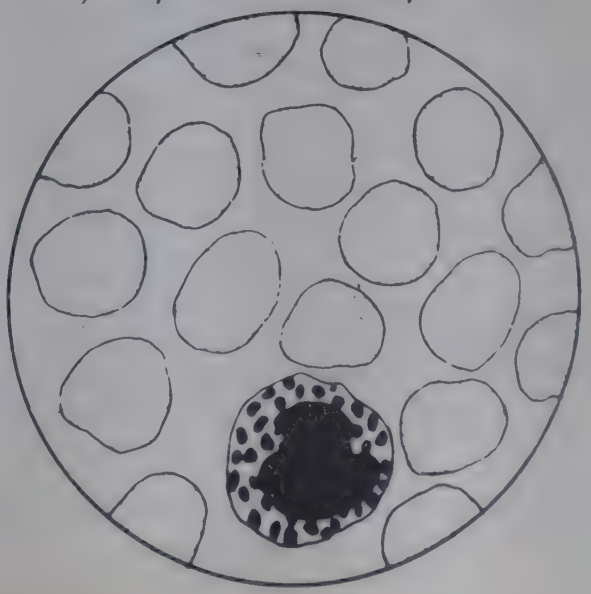
ii. *Polymorphonuclear eosinophil cell*



Size	: 12–15 μ
Granules	: large, round, orange-red, numerous and closely packed
Nucleus	: usually 2 lobes.

Sometimes the cell appears damaged, with scattered granules.

iii. *Polymorphonuclear basophil cell*

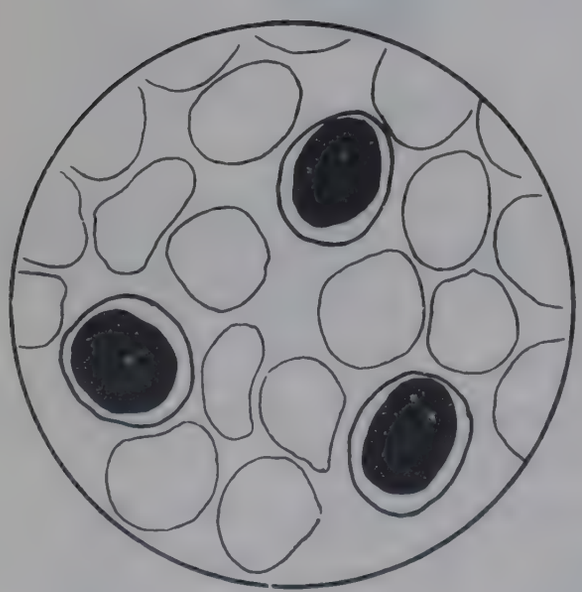


The rarest type of granulocyte.

Size	: 11–13 μ
Shape	: round
Granules	: very large, round, deep purple, numerous but less closely packed than those of the eosinophils
Nucleus	: difficult to see because covered by the granules
Vacuoles	: occasional small colourless vacuoles in the cytoplasm.

iv. *Lymphocyte*

(a) *Small lymphocyte*:

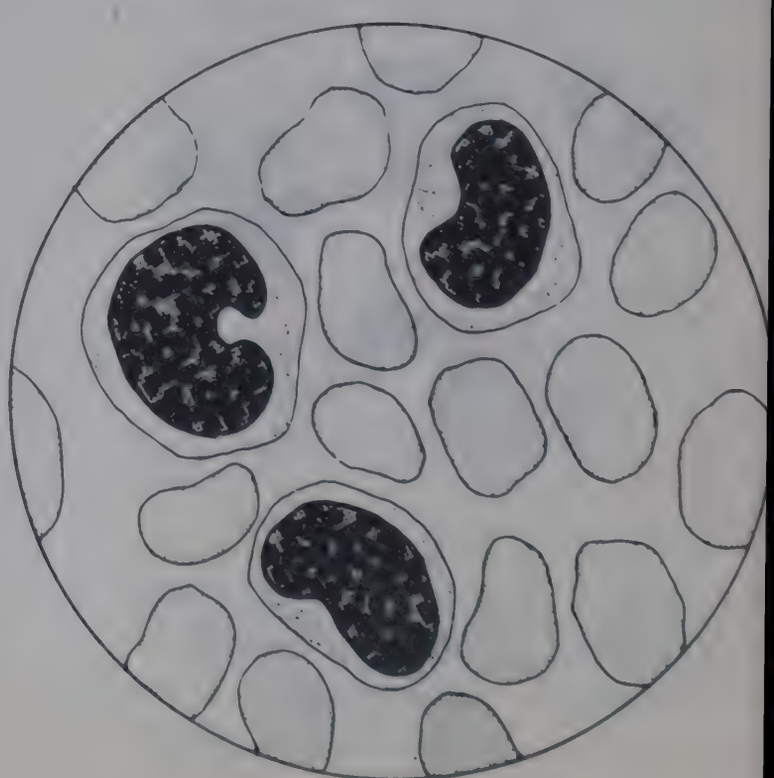


Size	: 7–10 μ
Shape	: round
Nucleus	: large, occupying most of the cell, chromatin dark purple, dense
Cytoplasm	: very little visible; blue with no granules.

(b) *Large lymphocyte*:

Size	: 10–15 μ
Shape	: round or irregular
Nucleus	: oval or round, may lie to one side of the cell
Cytoplasm	: abundant, pale blue
Granules	: few, quite large, azurophilic (dark red).

v. *Monocyte*



Size	: 15–25 μ; largest of the leucocytes
Shape	: irregular
Nucleus	: variable, often kidney-shaped, chromatin arranged in strands, pale mauve
Granules	: fine, dust-like, usually reddish
Vacuoles	: usually present in the cytoplasm.

In patients suffering from malaria the cytoplasm often contains brownish-black masses. This is malaria pigment.

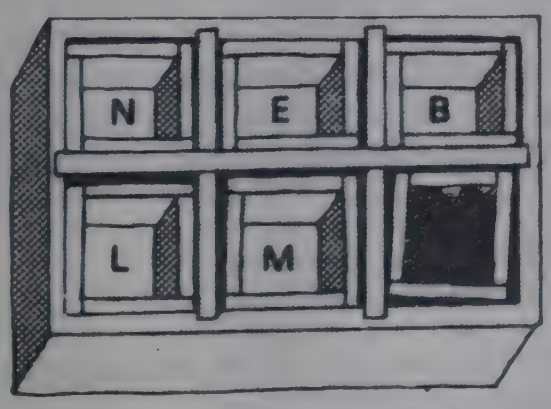
Counting methods

Using a bead counter box

A counting box like the one in the figure can be made locally. It consists of:

– 5 boxes labelled as follows:
  N = neutrophils
  E = eosinophils
  B = basophils
  L = lymphocytes
  M = monocytes

– a 6th box containing 100 beads (or beans or maize grains), used for counting.



Whenever you see a neutrophil, place a bead in the box marked N; whenever you see an eosinophil, place a bead in the box marked E; and so on. After all 100 beads have been used up, count the number of beads in each box.

Using a paper and pencil

Draw up a table with

– 5 vertical columns (N, E, B, L, M); and
– 10 horizontal lines

When 10 strokes have been made in the first line, go on to the next. Thus, when the 10th line has been completed, you know that you have counted 100 cells.

Then add up the total for each vertical column.

These totals give the percentage of each type of leucocyte.

	N	E	B	L	M
1	𝍸	||		|||	|
2	𝍸 |	|		|||	
3	𝍸 ||			|||	
4	||||	|	|	|||	|
5	𝍸 |||			||	
6	𝍸 ||	|		||	
7	𝍸			|||	||
8	𝍸 ||	|		||	
9	𝍸 |	|		|||	
10	𝍸	|		||||	
Total	59	8	1	28	4

Normal values

	Normal ranges for the following age groups (percentage)				
	Newborns	*After 4 days*	*1–4 years*	*10 years*	*Adults*
P. neutrophils	55–65	40–48	36–48	45–55	55–65
P. eosinophils	2– 4	2– 5	2– 5	2– 5	2– 4
P. basophils	0– 1	0– 1	0– 1	0– 1	0– 1
Lymphocytes	30–35	40–48	44–54	38–45	25–35
Monocytes	3– 6	5–10	3– 6	3– 6	3– 6

Abnormal findings

Neutrophilia:

An increased proportion of neutrophils (above 65%).

Particularly in acute infections.

Eosinophilia:

An increased proportion of eosinophils (above 5%).

Almost always suggests a parasitic worm infection localized in the tissues, e.g. filariasis, hookworm, ascariasis, etc. Can also be caused by an allergy.

Lymphocytosis:

An increased proportion of lymphocytes (above 35%).

Found in certain virus infections (measles, etc), certain chronic infections (malaria, tuberculosis, etc), and some toxic conditions.

Monocytosis:

An increased proportion of monocytes (above 6%).

Found in certain bacterial and parasitic infections such as typhoid fever, malaria and kala-azar.

Neutropaenia:

A decreased number of neutrophils.

May occur in certain infections and some other diseases

8. Identification of Malaria Parasites

The parasites that cause malaria are found in the blood; part of their development takes place within the red blood cells. Malaria parasites are detected in blood films stained by the JSB or Giemsa stain.

(1) When to collect the specimen

The parasites are usually most numerous in the blood towards the end of an attack of fever.

Always collect the blood *before* antimalarial drugs are given.

(2) Preparation of thick and thin blood films

Principle

A drop of blood from the finger is placed on a slide, spread and dried. During staining of the drop of dried blood, the haemoglobin in the red blood cells dissolves and is washed out by the water in the staining solution. All that remain are:

- the malaria parasites and
- the white blood cells

which can be seen under the microscope.

The thick film method makes it possible to find parasites:

- more quickly
- if there are only a few present.

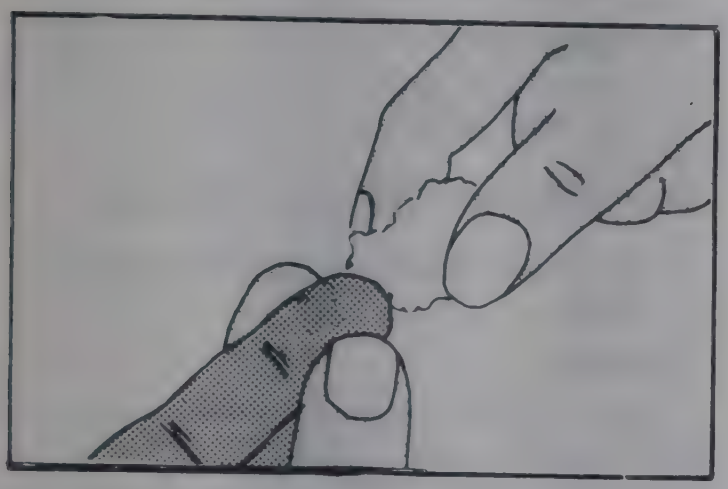
Materials

- Cotton wool
- Spirit or ethanol
- Hagedorn needle or sterile lancet
- Clean grease-free slides and spreader slide
- Lead pencil

Method

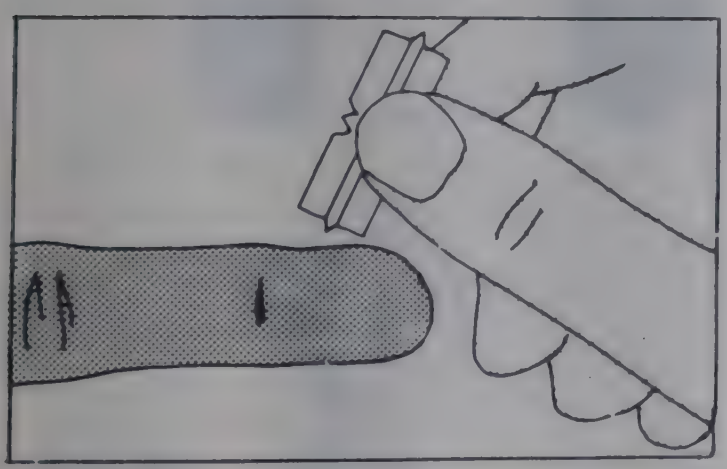
i. Hold the finger to be pricked.

ii. Clean the tip of the finger with spirit.



iii. Dry with another piece of cotton wool.

iv. Prick the finger at the side of the tip with the needle or lancet.



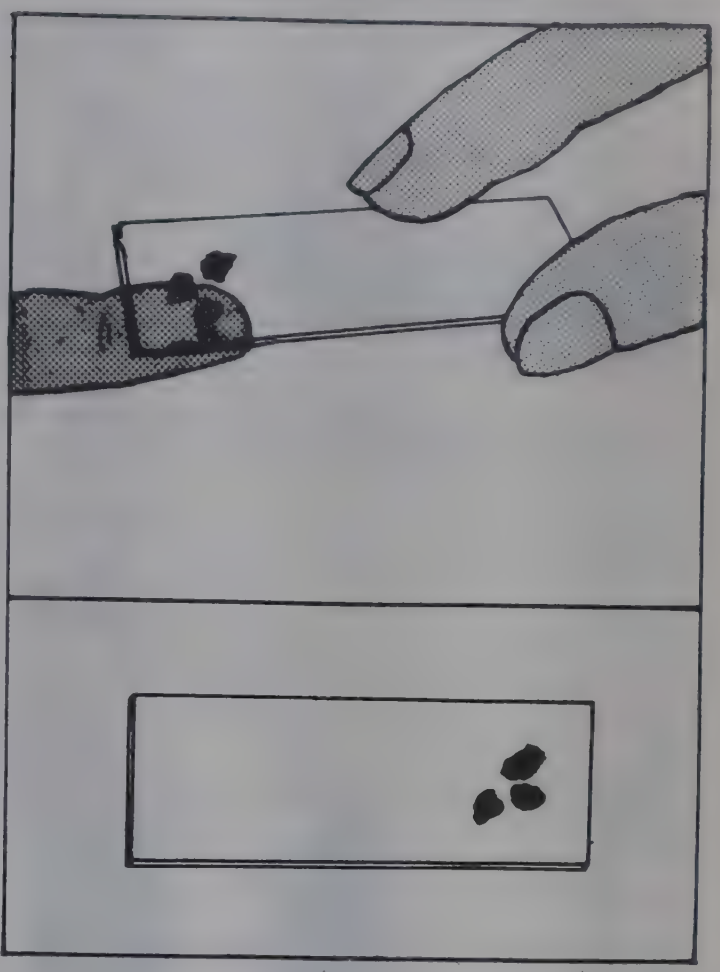
v. Allow the blood to flow freely.

vi. Discard the first drop of blood.

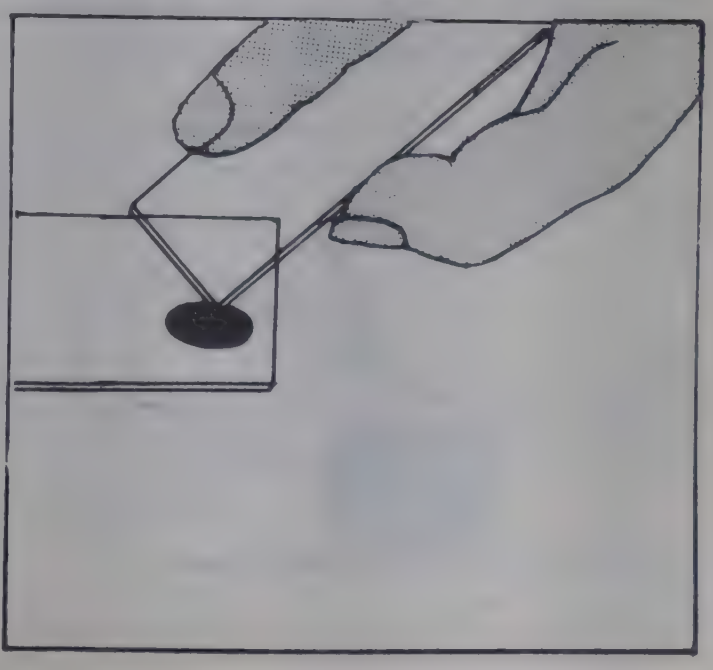
vii. The next blood drops should be collected for examination.

viii. *Thick film*:

– Put three drops of blood on the right hand quarter of the slide.

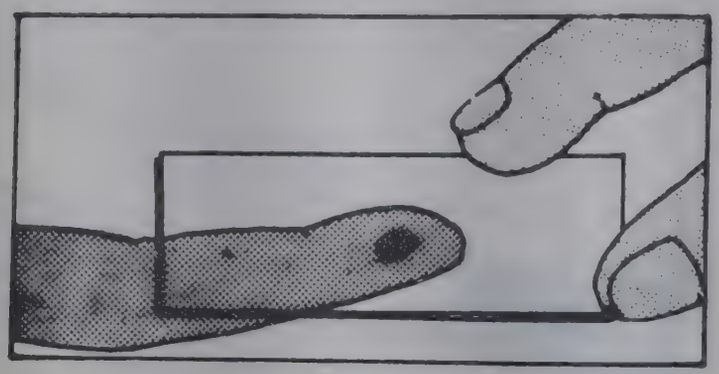


– With the corner of another clean slide, spread the blood to an even thickness in a round form of about 1 cm diameter.

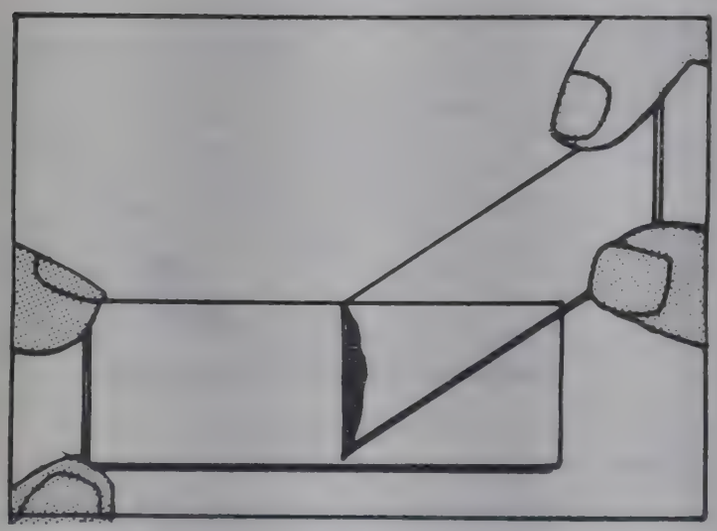


ix. *Thin film*:

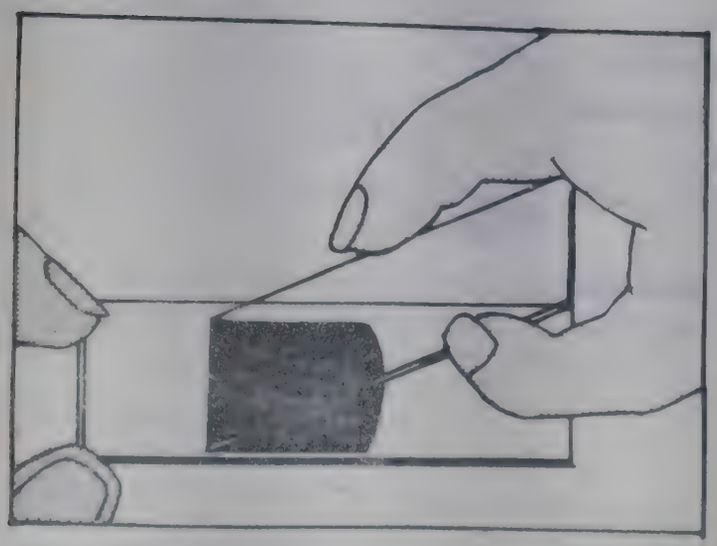
– Put a drop of blood on the middle of the same slide.



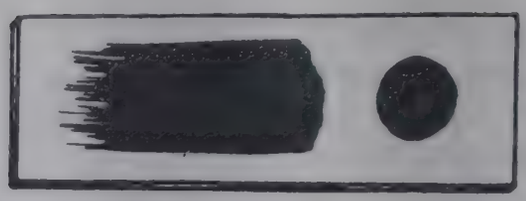
– Use the spreader slide and allow the drop of blood to spread along it.



– Push the spreader quickly from the centre to the left side of the slide, drawing the blood behind it.



x. Leave the film to dry.



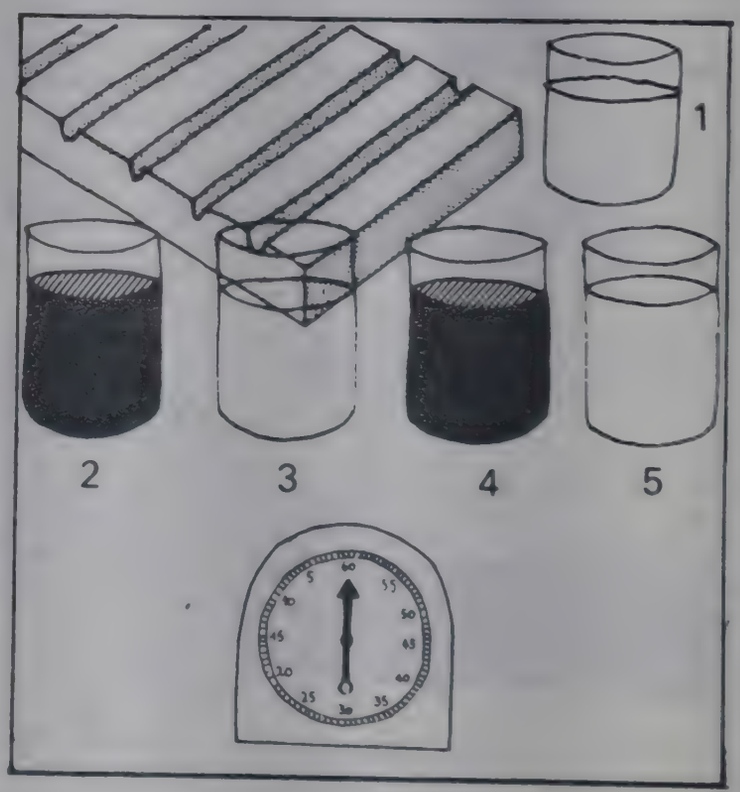
Do not blow on it or shake the slide. Protect the slide from flies and dust. An electric fan, if available, will speed up drying and keep flies away.

xi. When the film has dried, mark the slide by writing the patient's number on the end of the thin film, using a lead pencil.

(3) Staining of thick and thin blood films

A. By Jaswant Singh & Bhattacharya (JSB) Stain

Materials



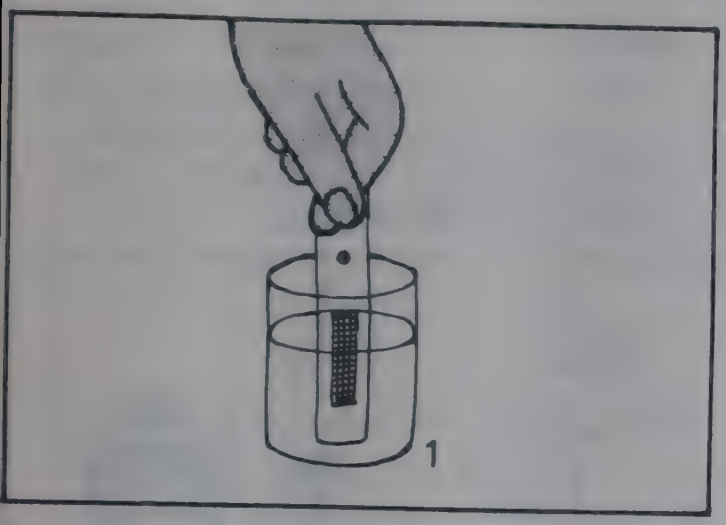
– 5 containers
– Draining rack
– Timer
– Jar No. 1 containing Methanol (methyl alcohol) – acetone-free

– Jar No. 2 containing JSB Stain Solution II (Reagent No. 17)
– Jars Nos. 3 & 5 containing Buffered water (Reagent No. 6)
– Jar No. 4 containing JSB Stain Solution I (Reagent No. 17)

Note: If scum forms on the top of the staining solution, filter the solution before using.

Method

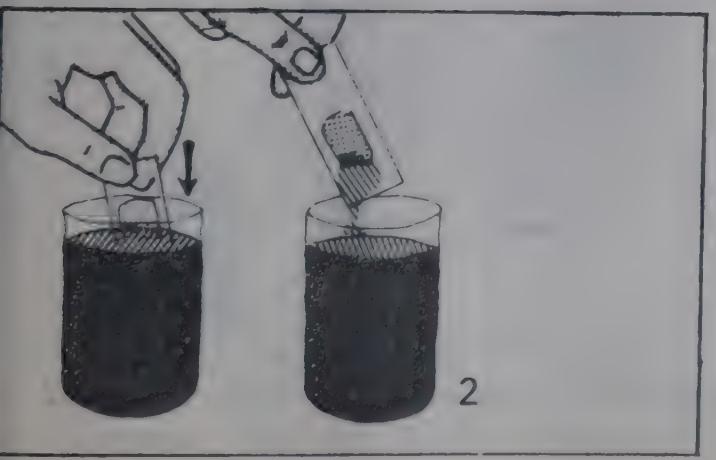
i. When both the thick and thin films are dried, immerse the *thin film only* in jar 1 containing methanol and rapidly remove it.



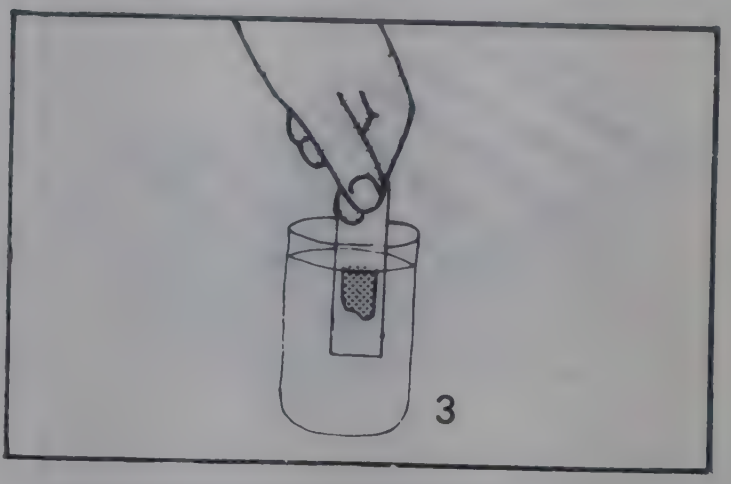
Take care not to let the methanol touch the thick film.

ii. Dry thoroughly in air.

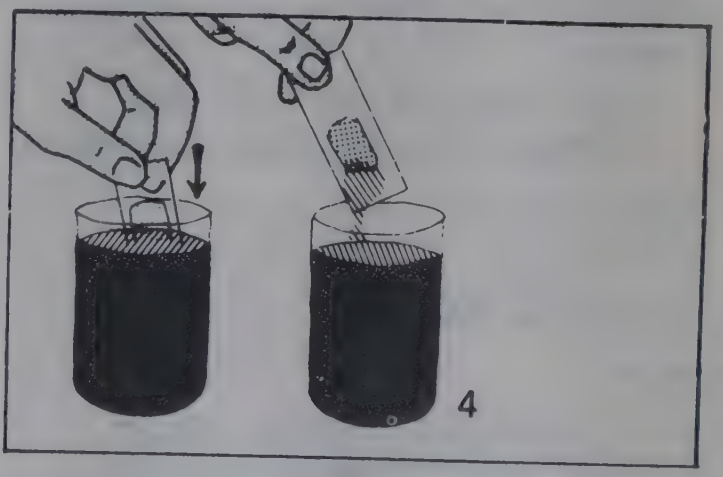
iii. Dip both the thick and thin films in jar 2 containing JSB Solution II for 1–2 seconds.



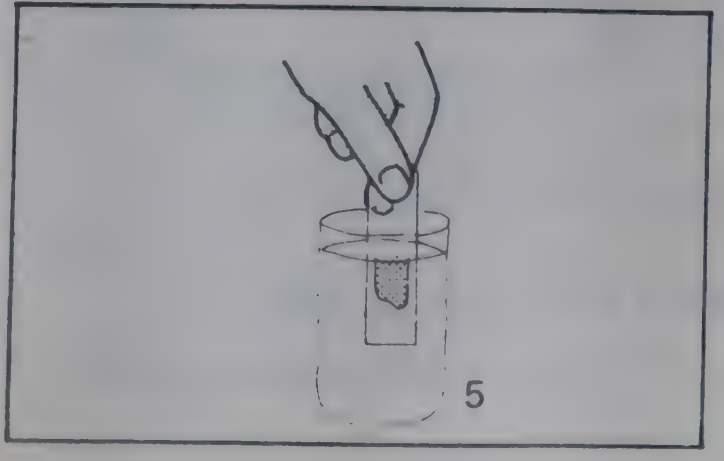
iv. Dip the slide twice or thrice in jar 3 containing buffered water to remove excess of eosin stain.



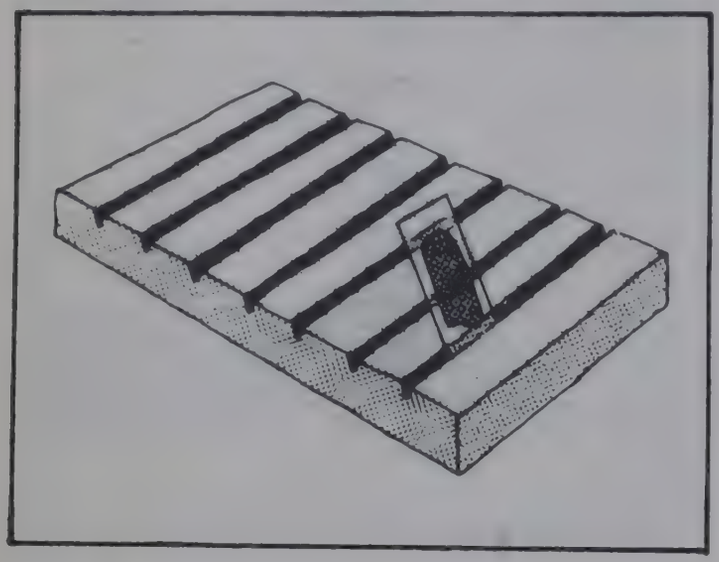
v. Imrnerse both thick and thin films in jar 4 containing JSB Solution I *for 45 seconds.*



vi. Dip the slide three or four times in jar 5 containing buffered water to remove excess of blue stain.



vii. Dry in air on a draining rack (with the side with the smear facing down).



The slide is now ready for examination. The smear should appear mauve. This will enable a malaria trophozoite to be recognized:

– the cytoplasm ring stains blue
– the chromatin dot stains dark red.

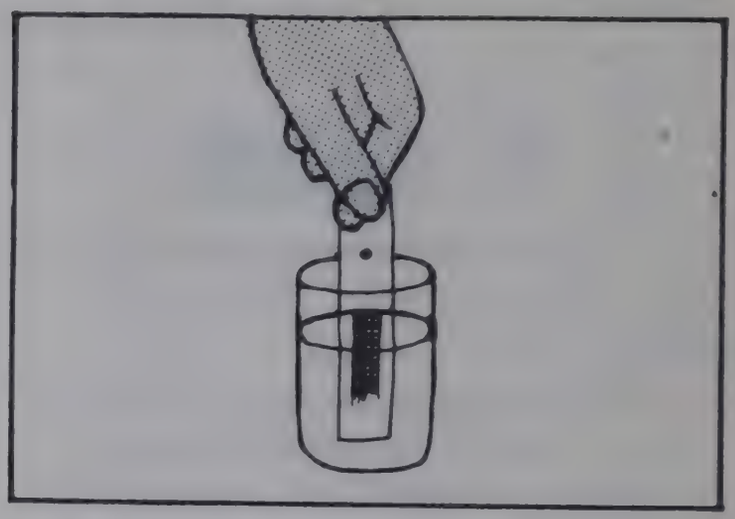
B. By Giemsa Stain

Materials

– 10, 50 and 100 ml measuring cylinders
– 50 and 250 ml beakers
– Dropping pipette
– Staining trough with cover
– Glass rods
– Wash bottle
– Slide forceps
– Slide rack
– Timer
– Giemsa stain (Reagent No.14)
– Jar containing Methanol
– Buffered water (Reagent No. 5)

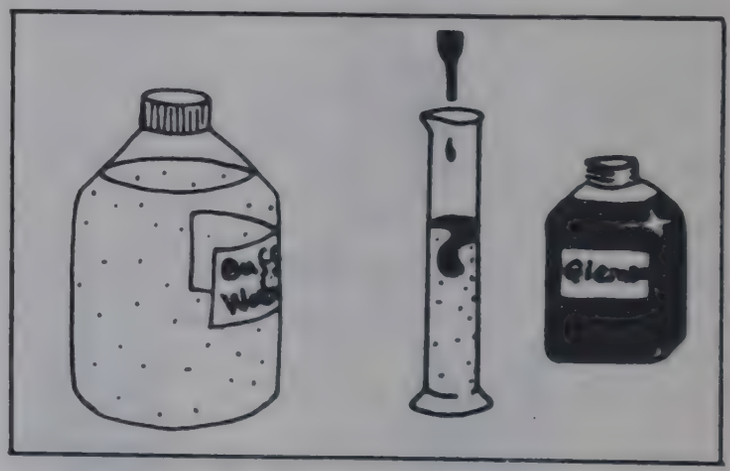
Method for less than 10 smears

i. Fix *thin film only* by dipping in methanol for 2–3 minutes.

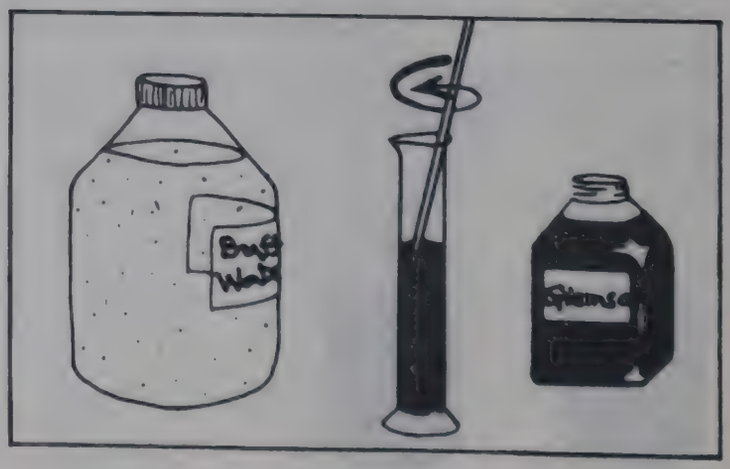


ii. Dry in air.

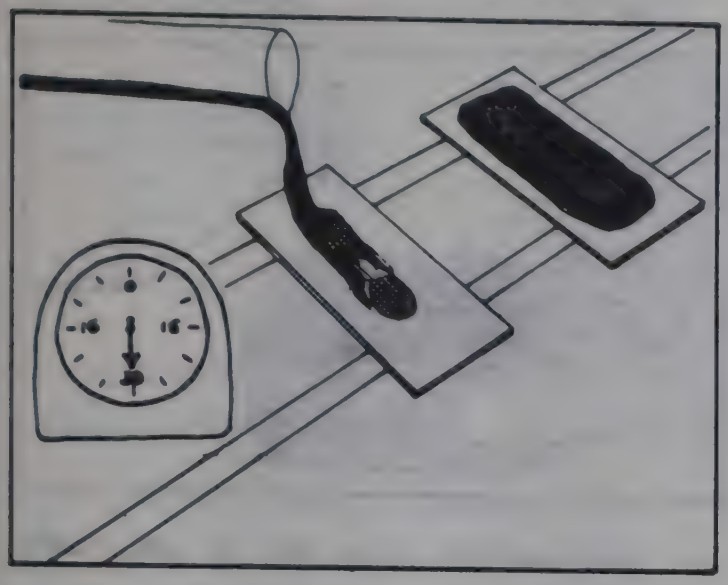
iii. Make a 1 in 10 dilution of Giemsa stain.
Example: Use 18 ml buffered water and 2 ml stain; this will be sufficient for 4 smears. Increase the volume if more smears are to be stained.



iv. Mix gently with a glass rod.



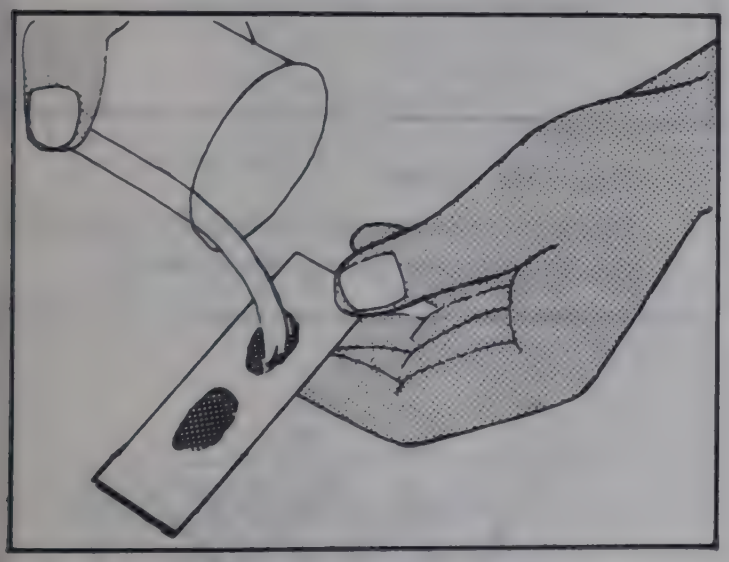
v. Place the slides across 2 glass rods. Cover them with diluted Giemsa stain.



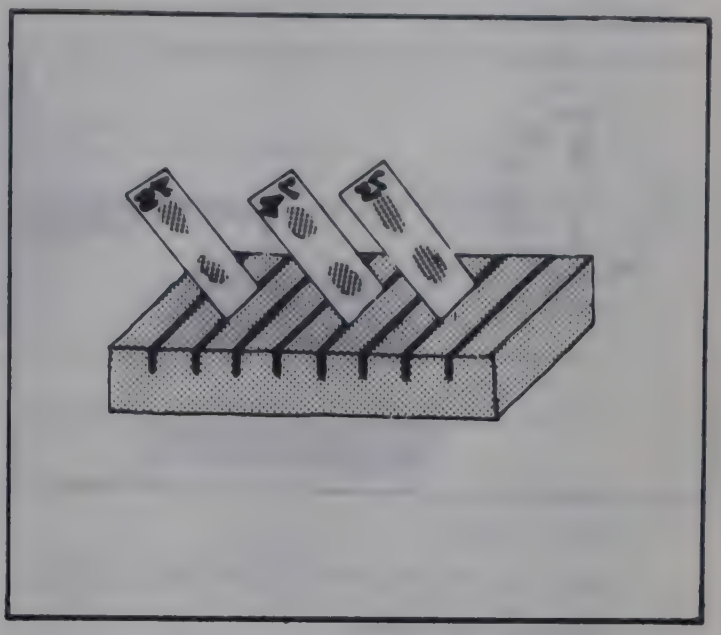
vi. Leave for 30 minutes.

Note: The staining time is given by the manufacturer. You may wish to adjust it when you have used the technique several times. If the film is too pale, the staining time has not been long enough.

vii. Wash off the stain with buffered water. Do not tip off the stain and then wash, as this will leave a deposit of stain over the smear.



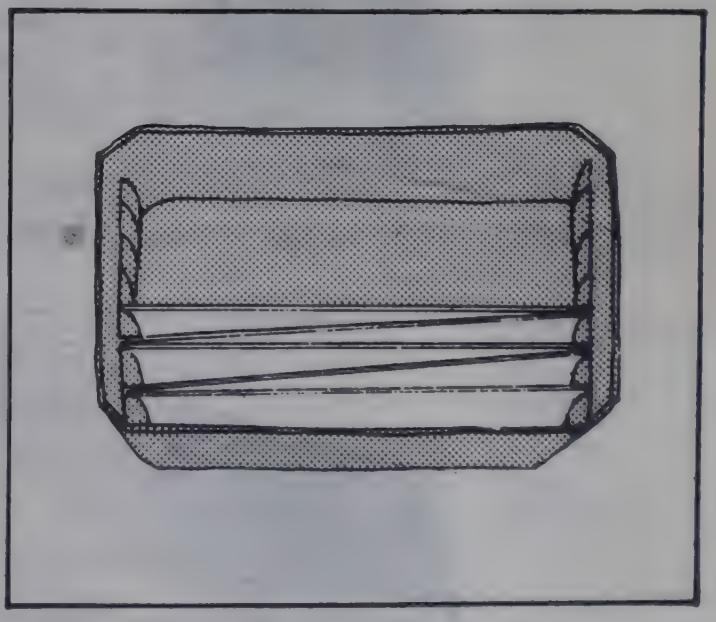
viii. Drain off the water. Place the slides in a rack to dry. Place them in a sloping position with the stained films facing downwards to protect them from dust in the air.



Drying stained slides by blotting them between sheets of filter paper is *not* recommended.

Method for large numbers of smears

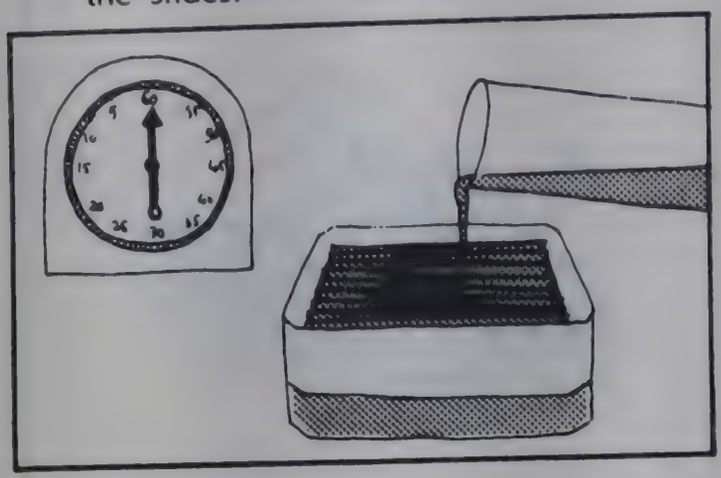
i. Using forceps, pick up the slides one by one and slot them into the rack of the staining trough, in a Z pattern.



Note: Thin films have first to be fixed for 2–3 minutes in methanol.

ii. Make up sufficient stain to fill the staining trough.

Slowly fill the staining trough containing the slides.

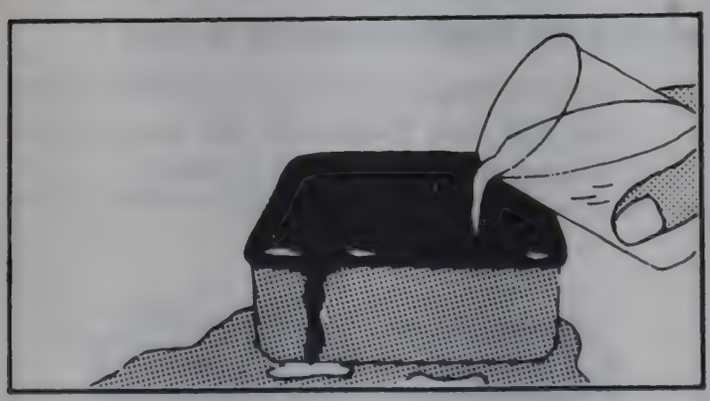


Cover.

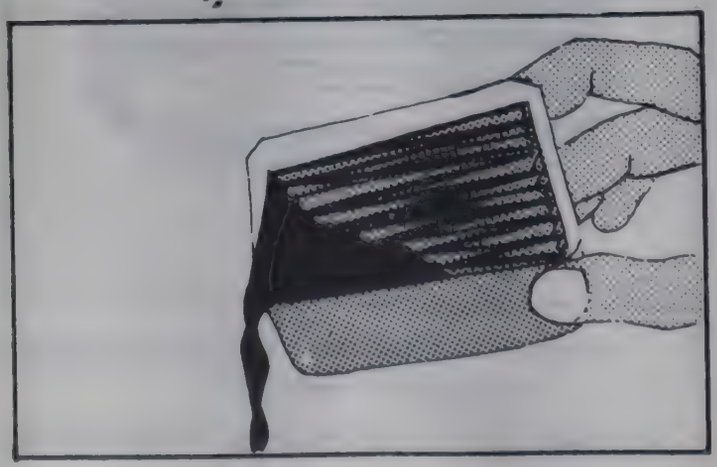
Leave for 30 minutes out of the sunlight.

iii. Remove the cover.

Slowly pour clean water from a beaker into the trough to remove the deposit on the surface of the staining solution.

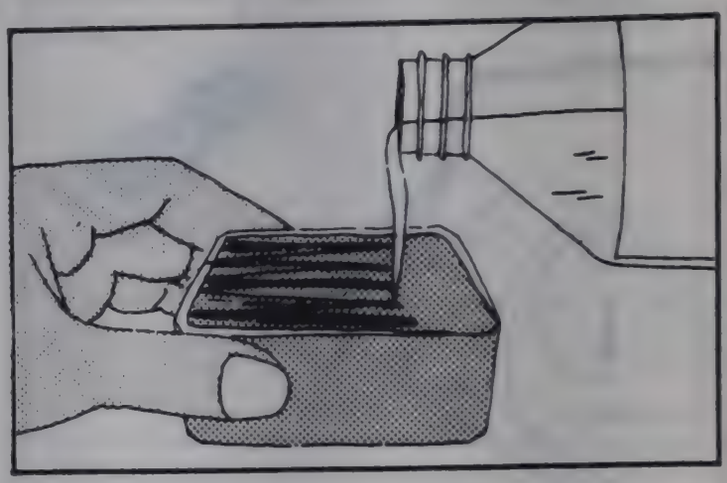


iv. Gently pour off all the staining solution from the trough.



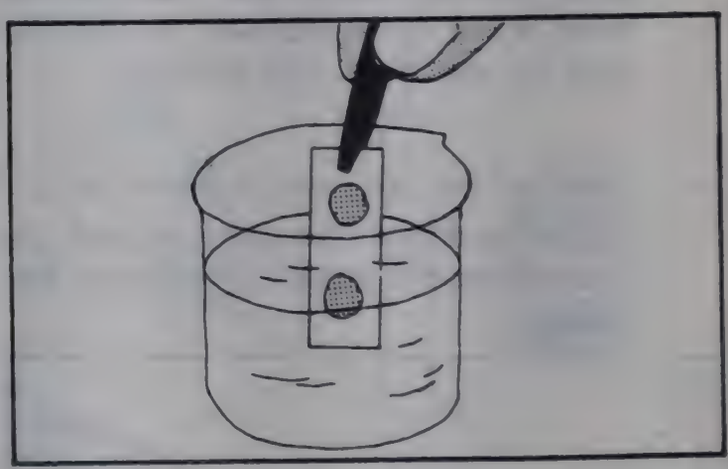
Note: If the dilute Giemsa is kept for re-use, it must be used on the same day.

v. Fill the staining trough with buffered water.



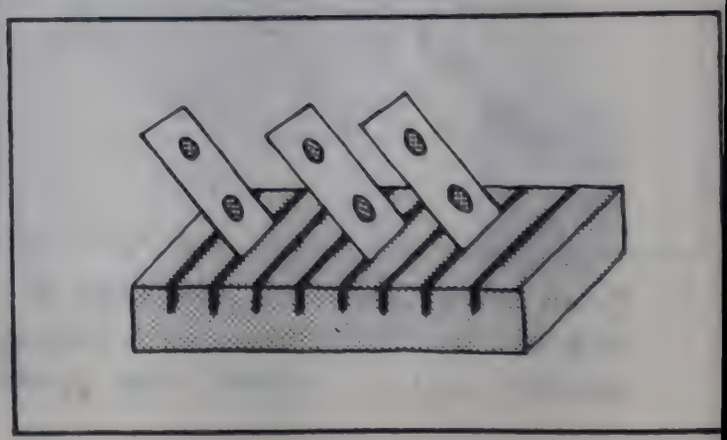
vi. Take out the slides one by one, using forceps.

Dip each slide in a beaker of ordinary water, gently, so that the stained preparation does not become unstuck.



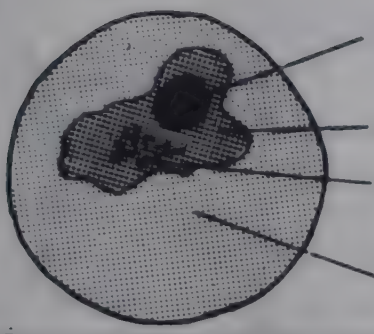
vii. Drain the slides.

Place them in the rack to dry (the side with the blood film facing downwards).



Malaria parasites

MALARIA PARASITE
(stained)



1 or more nuclei containing red chromatin

blue cytoplasm

occasional granules of black or brown pigment

the infected red blood cell (pink)

STAGES OF DEVELOPMENT Parasites found in the blood are at different stages of development

1. *Trophozoite*

Earliest stage with 1 nucleus living inside the red cell

young form

nucleus

thin ring of cytoplasm

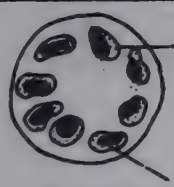
more compact cytoplasm

or

amoeboid cytoplasm

2. *Schizont*

Mature trophozoite with nucleus divided into 8–24 nuclei.
Fills most of the red cell



nuclei often arranged in a circle forming a rosette

each nucleus enclosed by some cytoplasm, forming a merozoite

3. *Gametocyte*

Sexual form with one large compact and round or elongated nucleus

male gametocyte

female gametocyte

Pigment

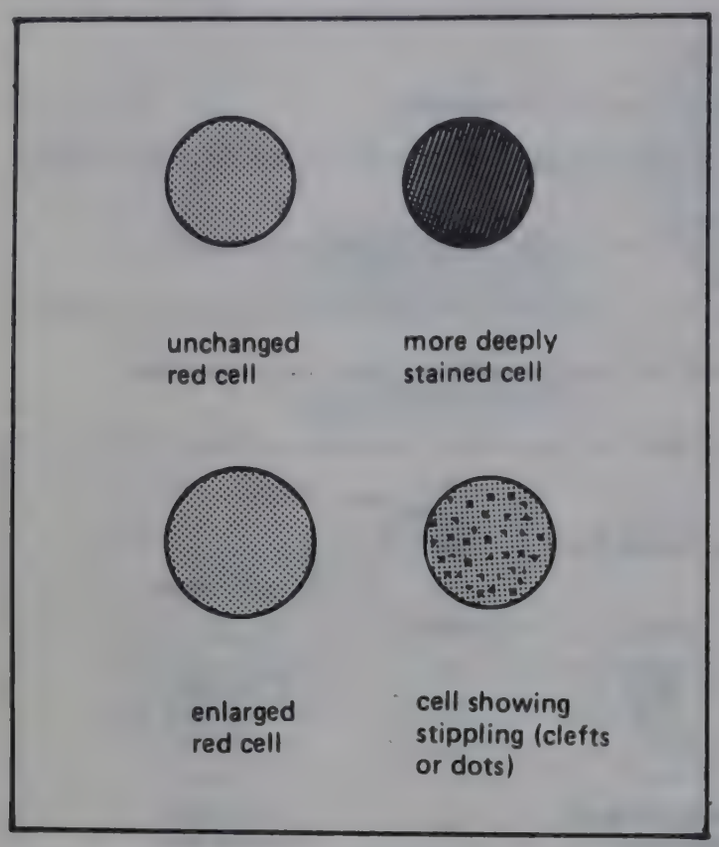
Some parasites have granules of pigment in their cytoplasm; others do not

with pigment

without pigment

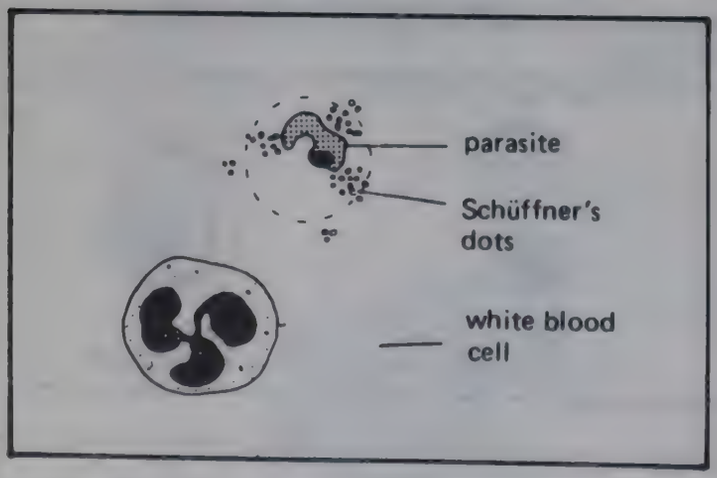
Infected red blood cells

In thin films:

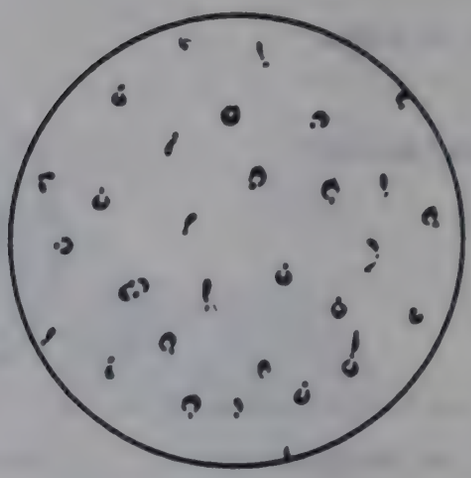


– the infected RBC may remain the same
– they may have changed colour or shape
– they may contain pink spots (Schüffner's dots).

In thick films:



– the red blood cells have practically disappeared
– the pink Schüffner's dots can still be seen around the parasite
– the leucocytes remain unchanged.



high density:
20 (or more) parasites per field

medium density:
2–19 parasites per field

low density:
1 (or less) parasite per field

Parasite density

This is the number of parasites counted in each microscopical field.

It usually varies according to species and it might therefore be useful to consider the density *in the thick film.*

It is important to report the parasite density. When the parasites are very numerous (very high parasite density), the patient requires urgent treatment. Therefore, if you find a high parasite density, state the result clearly in your report and send it without delay to the physician.

Parasite species

There are three main species of human malaria parasite:

- Plasmodium falciparum
- Plasmodium vivax
- Plasmodium malariae

It is important for the prognosis and treatment of the disease that the species involved be identified in the laboratory. However, if you cannot identify the species, always report the presence of any malaria parasites you see.

For example:

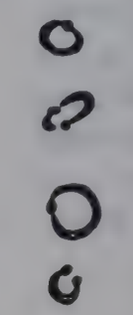
- Malaria caused by P. falciparum is much more serious than malaria caused by P. malariae and sometimes causes death.
- If not properly treated, however, a P. malariae infection can last much longer than a P. falciparum infection.

A patient can harbour more than one species of malaria parasite at the same time (mixed infection).

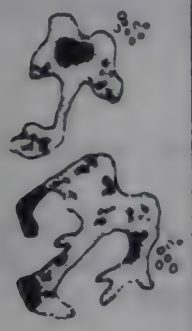
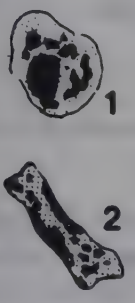
For instance:

- Plasmodium falciparum and Plasmodium malariae
  or
- Plasmodium falciparum and Plasmodium vivax

Identification of the different species of malaria parasites in blood films

	PLASMODIUM FALCIPARUM		*PLASMODIUM*
YOUNG TROPHOZOITE	(Stage frequently found) *Cytoplasm:* small fine pale blue ring *Chromatin:* 1 or 2 small red dots		(Stage frequently found) *Cytoplasm:* irregular blue quite thick ring *Chromatin:* 1 rather large red dot
MATURE TROPHOZOITE	(Stage frequently found) *Cytoplasm:* rather thin blue ring, or shaped like a comma or exclamation mark *Chromatin:* 1 or 2 medium-sized red dots		(Not frequently found) *Cytoplasm:* large, blue, irregular (sometimes divided into 2, 3 or 4); small particles of brownish-orange pigment *Chromatin:* 1 red dot
SCHIZONT	(Very rare) Hardly ever found in blood films (except in very serious cases) *Merozoites:* 18–32 *Pigment:* dark brownish-black		(Quite frequently found) *Merozoites:* 12–18 large compact red granules seen against the pale blue cytoplasm

Identification of the different species of malaria parasites in blood films (contd.)

VIVAX	*PLASMODIUM MALARIAE*	
	(Stage frequently found) *Cytoplasm:* thick, dense, blue ring with some granules of black pigment *Chromatin:* 1 large red dot	
	(Stage frequently found) *Cytoplasm:* either (1) round, compact, dark blue, with many black particles of pigment, or (2) in band form (in thin films only) *Chromatin:* a round dot or a red band	 1 2
	(Fairly frequently found) *Merozoites:* 8–10 Each one a large red spot enclosed by pale cytoplasm; the 8 spots may be arranged irregularly (young form) or in a rosette *Pigment:* always seen	

Identification of the different species of malaria parasites in blood films (contd.)

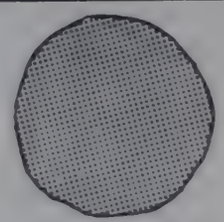
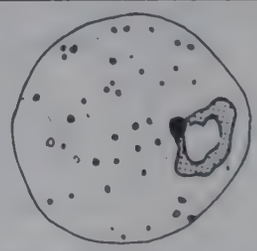
	PLASMODIUM FALCIPARUM		*PLASMODIUM*
GAMETOCYTE	(Fairly frequently found) *Shape:* like a banana or sickle *Colour:* blue (male) or dense blue (female) *Nucleus:* reddish-pink *Pigment:* a few blue-black granules in the centre of the cytoplasm or scattered through it		(Frequently found) *Female:* oval or rounded, dense blue A dense red triangular nucleus, often at one end; many particles of orange pigment in the cytoplasm *Male:* rounded, pale blue A round central pale red nucleus; some particles of orange pigment in the cytoplasm
RED CELLS	Normal in size May show crenation cells containing mature trophozoites; often contain a few red dots, irregular in size and shape		Enlarged, often pale-staining Schüffner dots, especially around mature trophozoites
*PARASITE DENSITY	often very high density		Medium density

* The parasite density in any area depends mainly on whether the malaria is seasonal or endemic, Adults, especially, build up immunity in endemic areas and the parasite density is often low.

Identification of the different species of malaria parasites in blood films

VIVAX	*PLASMODIUM MALARIAE*	
 	(Fairly frequently found) *Shape:* large, oval or rounded *Colour:* dense blue (female) or pale blue (male) *Nucleus:* 1 round spot of red chromatin against one edge *Pigment:* large black granules in the cytoplasm	 
	Normal in size and shape No red dots usually seen	
	Low density	

Comparison of infected cells in the thin blood film

	P. falciparum	*P. vivax*	*P. malariae*
SIZE of young trophozoite in comparison with diameter of red cell (at the same stage of development)	 1/5 to 1/3 of diameter	 1/4 to 2/3 of diameter	 1/4 to 2/3 of diameter, but often band form seen
APPEARANCE of infected red cell	 Remains unchanged	 Enlarged and often pale-staining	 Remains unchanged or becomes smaller and sometimes more deeply coloured
DOTS in the infected red cell	 Often none	 Small pink, Schüffner dots	 None
STAGES found	Trophozoites or gametocytes or both together; many trophozoites can be found in one cell	All forms found in the same film	All forms found in the same film

Reporting results

Positive result:

Specify : – The species of parasites found
– The stage of development of the parasite
– The parasite density.

Example :
Examination for malaria parasites positive.
Plasmodium falciparum – many trophozoites; a few gametocytes.
Parasite density: medium.

Negative result:

State: No parasites found.

Note: Do not mistake platelets superimposed upon red blood cells for malaria parasites.

9. Identification of Microfilariae

(1) Collection of specimen

Principle

Mix a fresh smear of capillary blood from the finger with sodium chloride solution, place between a slide and coverslip, and examine for motile microfilariae under the low power of the microscope.

Time of collection

The examination should be carried out at night. Some species of microfilariae appear in the blood during the day also.

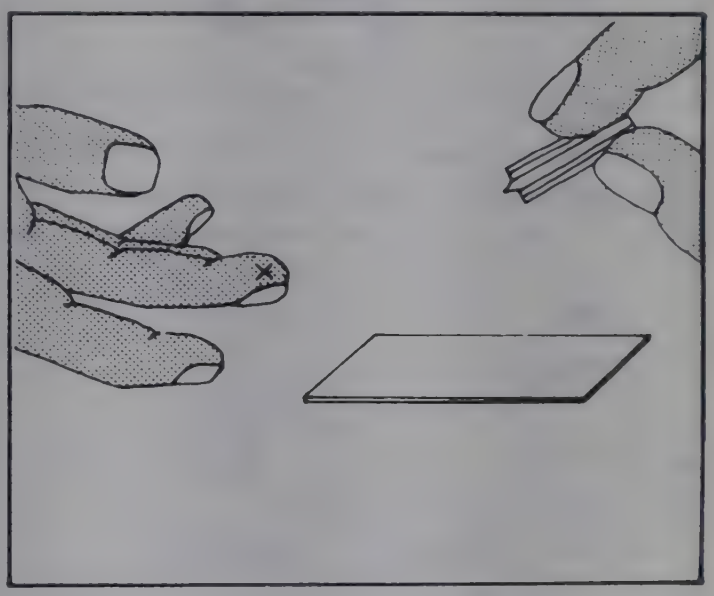
Species occurring in India	*When to take a specimen*
Wuchereria bancrofti	At night, between 10 p.m. and 4 a.m
Brugia malayi	Mainly at night, between 10 p.m. and 4 a.m.

Materials

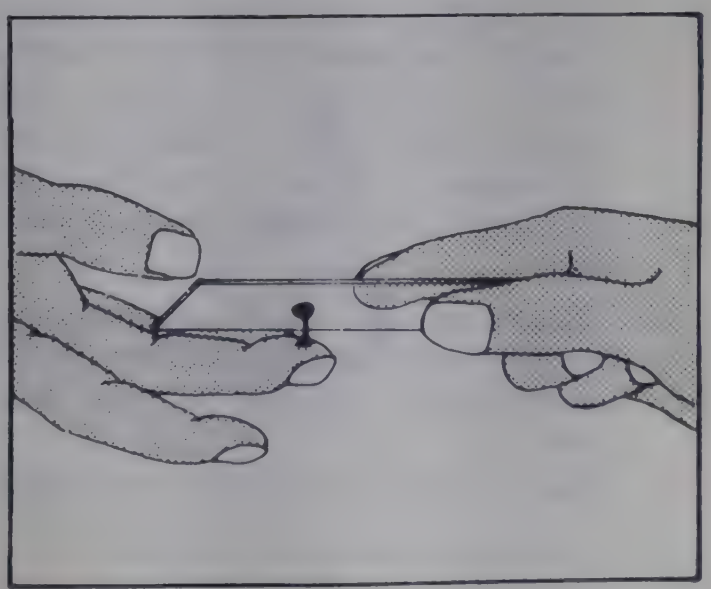
– Blood lancet
– Cotton wool swabs
– Ethanol
– Clean slides
– Coverslips
– Grease pencil for marking the slide
– Sodium chloride solution (Reagent No. 25)

Method

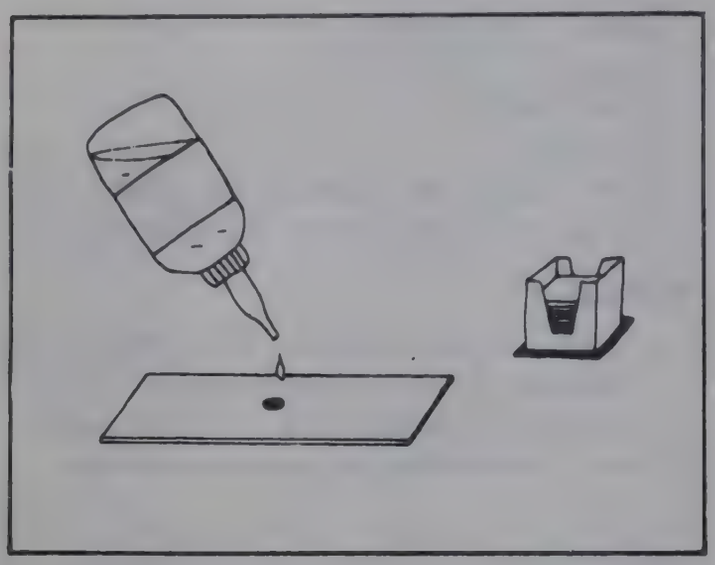
i. Sterilize the finger to be pricked with ethanol. Choose the third finger of the left hand. Dry well. Prick with the lancet.



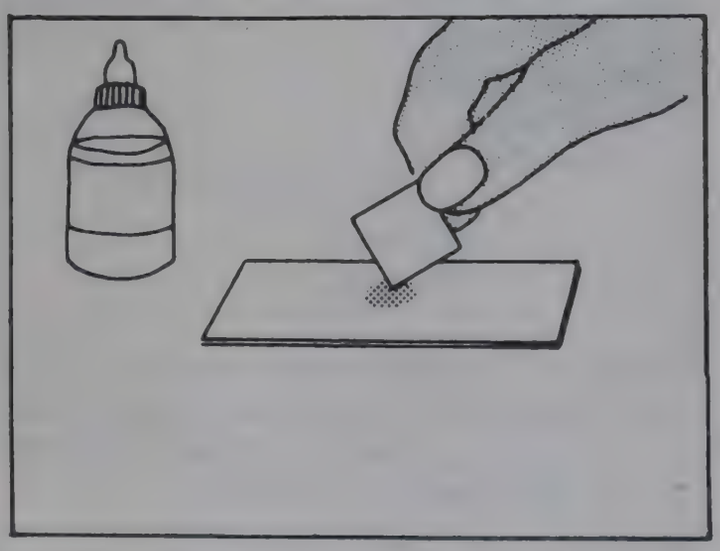
ii. Collect the *first drop* of blood that appears (it contains more microfilariae) directly on to the middle of the slide.



iii. Add an equal drop of sodium chloride solution.



iv. Mix the blood and sodium chloride solution using the corner of a coverslip.

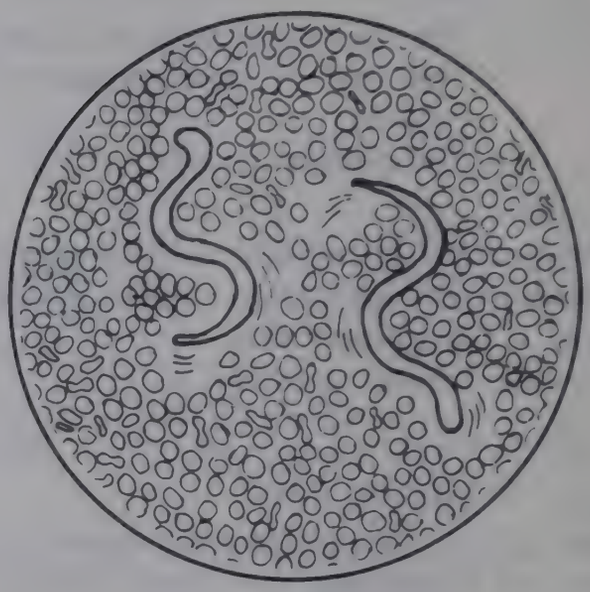


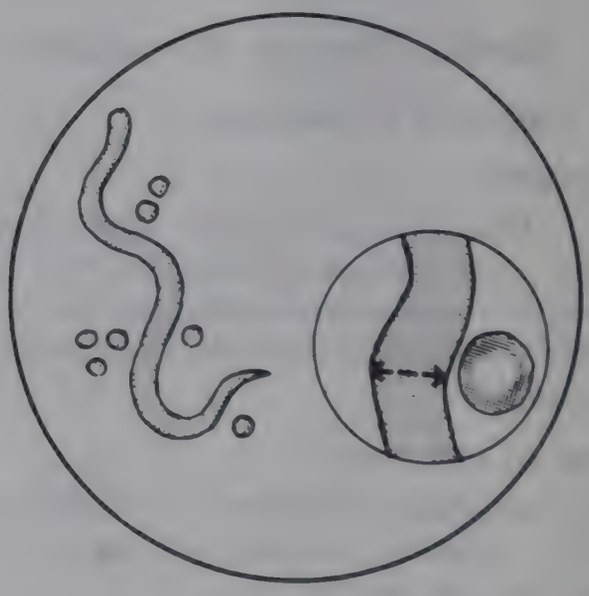
Cover the preparation with the coverslip.

v. Prepare 2 thick films on another slide using 2 drops more of blood, for the identification of stained microfilariae. (See page 147 for how to prepare a thick blood film.)

vi. Examine the fresh smear immediately and systematically under the microscope (× 10 objective with reduced condenser aperture).

The first sign of the presence of microfilariae is rapid movement among the red blood cells.





Note: Microfilariae are identified in stained smears.

It is possible, however, to gain some indication of the presence of microfilariae from a fresh blood smear.

(2) Staining of slides

Materials

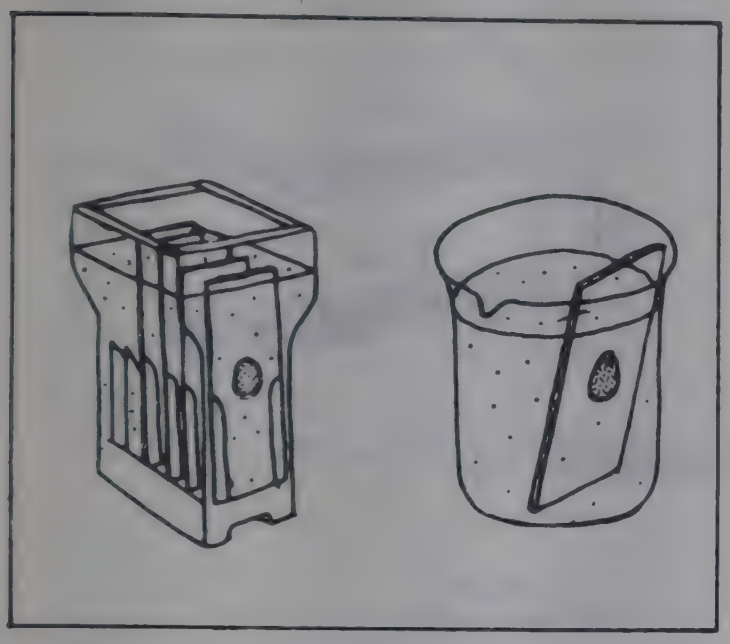
– Vertical staining trough with cover *or* beaker

– Materials required for Giemsa stain (see page 150)
– 2% Acid alcohol (2 parts commercial HCl and 98 parts methyl alcohol)

Method

i. *Removal of haemoglobin*:

(a) Place the slides vertically in the staining trough filled with clean water and cover. If no trough is available, use a beaker.



(b) Leave for 10 minutes (the haemoglobin sinks gradually to the bottom).

(c) Take the slides out and drain them.

(d) Fix the slides in 2% acid alcohol (one dip only).

ii. *Staining with Giemsa stain*:

Stain for 30 minutes using a 1 in 10 dilution of Giemsa as described for staining of thick blood film for malaria parasites (page 150).

(3) Microscopical examination

i. Cover the stained smear with a thin film of immersion oil.

ii. Look for microfilariae using the × 10 objective. They should stand out well.

iii. Examine the microfilariae found with the × 100 oil immersion objective.

Identification of W. bancrofti and B. malayi

Study in order of importance:

i. *Size*:

– 6 to 8 μ (as thick as one white blood cell.
– 200 to 300 μ in length

W. bancrofti:
the cephalic* space is as long as the breadth of the parasite

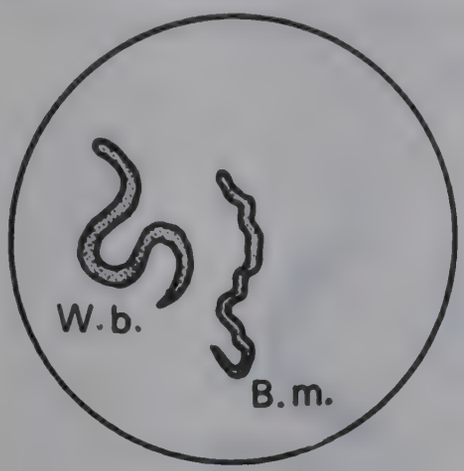
B. malayi:
the cephalic space is 1.5 times longer than the breadth of the parasite

(* at the head end)

ii. *Sheath*:

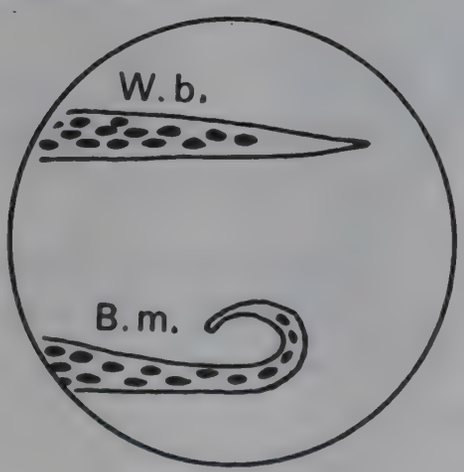
– Stains pink
– Length 350 μ extending beyond the two extremities

iii. *Body curves*:



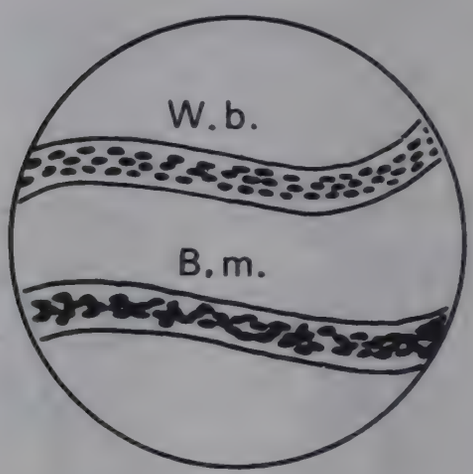
– W. bancrofti:
large curves
– B. malayi:
many small curves

iv. *Tail and its nuclei*:



– W. bancrofti:
straight tapered tail
nuclei not extending to tip of tail
– B. malayi:
curved tapered tail
two widely spaced nuclei at tip of tail

v. *Nuclei in the body*:



– W. bancrofti:
well separated
– B. malayi:
overlapping

Comparison of W. bancrofti and B. malayi (Giemsa stain)

	Wuchereria bancrofti	*Brugia malayi*
When found in blood	At night	Chiefly at night
Length	200–300 μ	220–250 μ
Thickness	8 μ (about 1 leucocyte) 	6 μm (almost 1 leucocyte) 
Sheath	Pink sheath 	Very pink sheath 
Body curves	Regular, large 	Small, irregular and many 
Tail	No nuclei at end**  Rather straight tapered tail	2 widely spaced nuclei at the end of tail  Curved very tapered tail
Body nuclei	Round medium-sized nuclei, well separated	Small angular nuclei, squeezed together and not very distinct

*In thick films the leucocytes always shrink and measure 8-11 μ

**The tail is sometimes broken or coiled, giving a false impression that there are nuclei right to the tip.

10. Erythrocyte Sedimentation Rate (ESR)

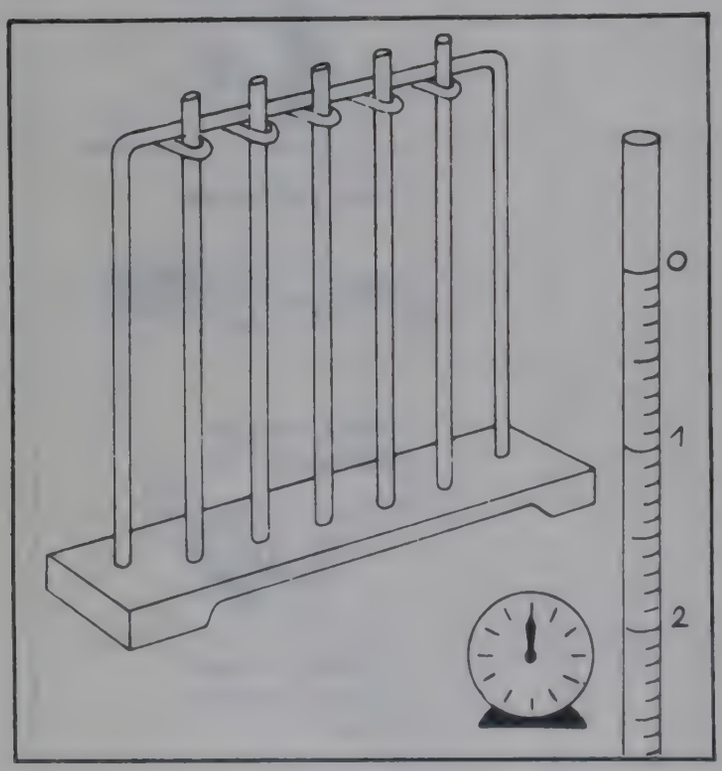
Principle

Blood collected into an anticoagulant is placed in a long graduated tube held in a vertical position.

The red blood cells settle to the bottom leaving a layer of plasma above.

The height of the column of plasma after 1 hour indicates the sedimentation rate of the erythrocytes (red blood cells).

Materials



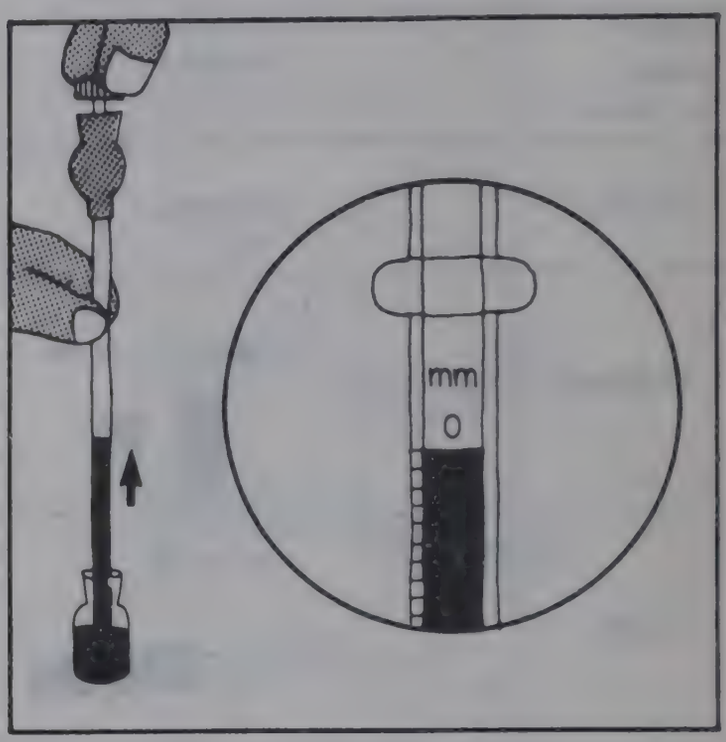
- Westergren ESR tube:
  *internal diameter 2.5 mm
  *graduated from 0 to 200 mm (often marked 1 to 20, 1 corresponding to 10, 2, to 20, etc.)
- Westergren stand
- Timer
- Rubber bulb

Method

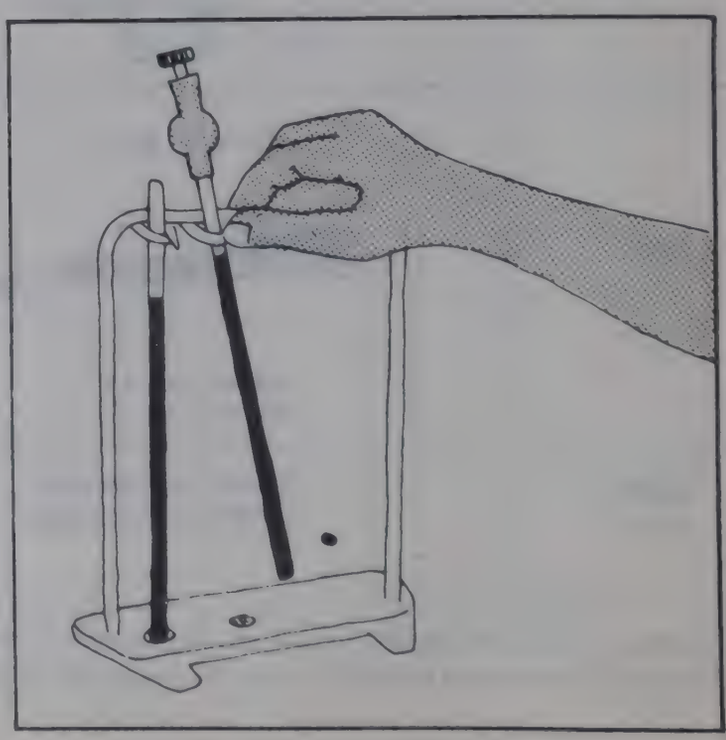
(1) Two ml venous blood collected in 0.4 ml of trisodium citrate solution (or EDTA dipotassium salt) is used for this test.

(2) Measurement of the ESR should begin within 2 hours of collection of the blood.

(3) Draw the citrated blood into the Westergren tube (using a rubber bulb if possible) up to the 0 mark.



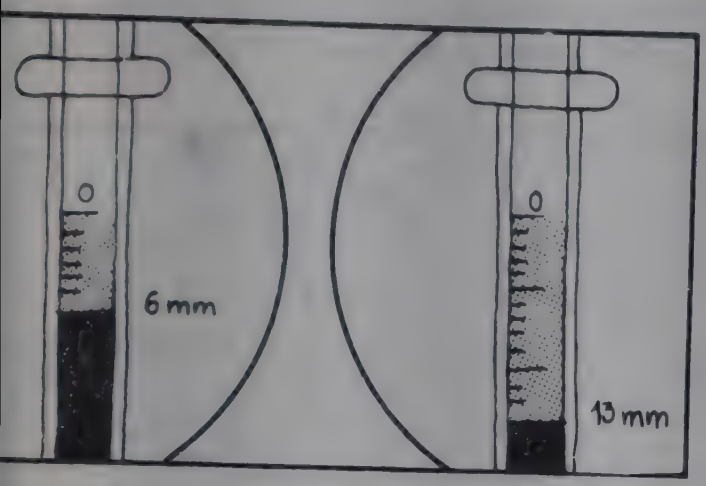
(4) Place the tube in the Westergren stand, making sure that the tube is completely upright.



Check that there are no air bubbles in the tube.

Check that the stand is level.

5) *Wait one hour* (set the timer to ring), then note the height of the column of plasma in mm graduations starting from the 0 mark at the top of the tube.



esults

he result is expressed as follows:
ESR . . . mm/h

Normal range

Men : 1–10 mm/h
Women : 3–14 mm/h

In normal pregnancy the ESR is raised.

Very high ESR values occur in:

- tuberculosis
- malignant diseases

Note:

- If the patient has a deficiency of red blood cells (anaemia) measurement of the ESR is of little value.
- If a patient is dehydrated measurement of the ESR has little value.
- The ESR increases with the temperature (from 23°C). Make sure that the stand is not placed in a warm part of the laboratory (e.g. not in the sun).

Washing Westergren tubes

- Rinse in water, then leave to soak in clean water for 12 hours.
- Dry completely (at 37°C if possible).
- Do not use washing powder, acids ethanol.

CHAPTER 10

Examination of Sputum

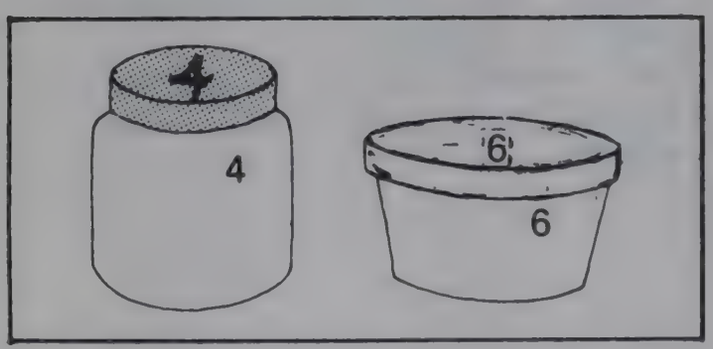
Sputum is the material derived from the lungs, bronchi, larynx and pharynx. It is brought up on coughing. It is always mixed with a varying quantity of saliva.

If anyone has a cough lasting for two or more weeks, his/her sputum should be collected and examined for the presence of tuberculosis bacilli.

1. Containers for Collecting Sputum Specimens

Glass screw-top jars *or* waxed paper cups (icecream cups) with lids can be used. The latter are preferable as they can be burned.

The patient's number should be written on the cup as well as on the lid.



2. Collection of Sputum Specimen

The quality of the sputum collected is very important.

Collect the first sputum of the morning.

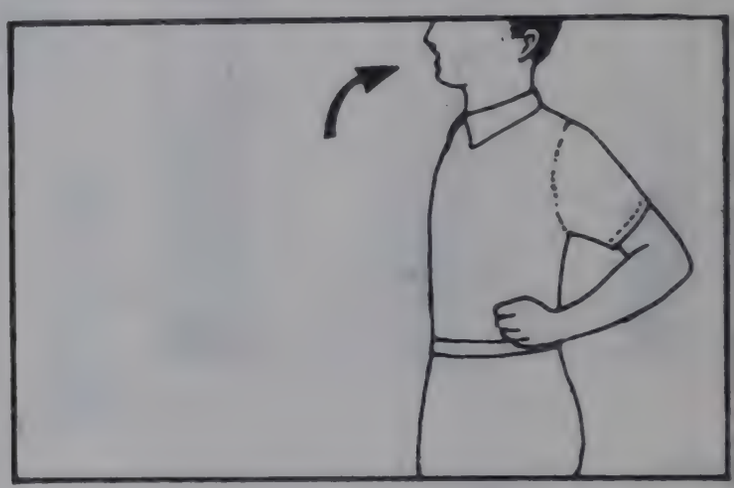
The Health Worker or Laboratory Technician must be present and the procedure described below should be followed:

(1) Sputum should be collected in an open space. The patient should face the wall away from the breeze.

(2) The patient should be standing, if possible.

(3) He should rinse his mouth so that the specimen is not mixed with food particles, betel or tobacco.

(4) He should place his hands on his hips, and take a very deep breath, filling his lungs.



(5) He should empty his lungs in one breath, coughing as hard and deeply as he can. He should collect the sputum in his mouth.

(6) He should lean forward slightly and gently spit what he has coughed up into the sputum cup.

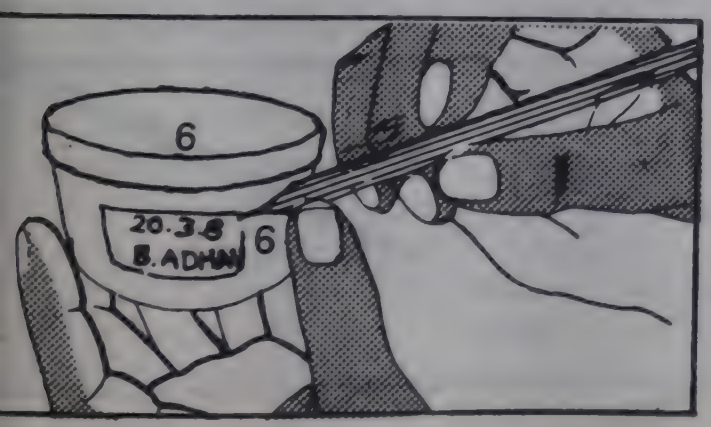
(7) He should repeat steps 4 to 6 so that a sufficient amount of solid or purulent material is collected and not merely saliva.

(8) Check that a sufficient amount of sputum has been produced. The sputum of an infected person usually contains:
- thick mucus with air bubbles
- threads of fibrin
- patches of pus
- occasional brownish streaks of blood.

Liquid frothy saliva and secretions from the nose and pharynx are not acceptable expectorations. Have the patient produce another specimen.

(9) Take care that the sputum is collected without soiling the outside of the sputum cup. If soiling does take place, prepare the smear immediately, burn the sputum cup, and wash the hands with soap and water.

(10) Cover the cup and label it clearly with the patient's name and the date.



3. Appearance of Sputum

Examine the sputum specimen for the following:

(1) **Quantity**
- Scanty
- Moderate
- Profuse

(2) **Consistency**
- Serous
- Frothy
- Mucoid
- Purulent
- Sero-purulent
- Haemorrhagic

(3) **Odour**
- Odourless
- Foul-smelling

(4) **Colour**
- Colourless and translucent
- Whitish yellow or greenish (pus)
- Blackish (carbon particles)
- Presence of bright red streaks (fresh blood)
- Dark brown or rusty (old blood)

Record any positive findings in the Sputum Report (See Appendix 5.3D).

4. Preparation of Sputum Smears

Principle

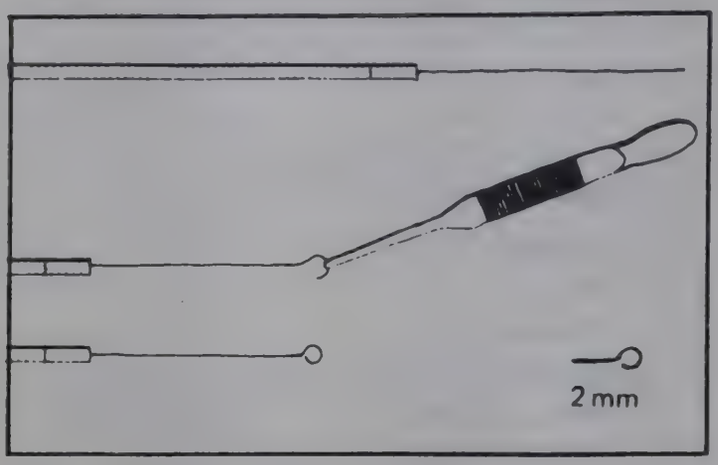
The sample to be examined is treated as follows:
- It is spread in a thin layer on a glass slide.
- It is dried completely.
- It is fixed on the slide by heating before being stained.

Materials

- Wire loop: This is a metal wire (usually made of nickel-chromium alloy) fixed on to a handle and bent into a loop at the other

end. Make the loop with forceps, taking care that it is centred.

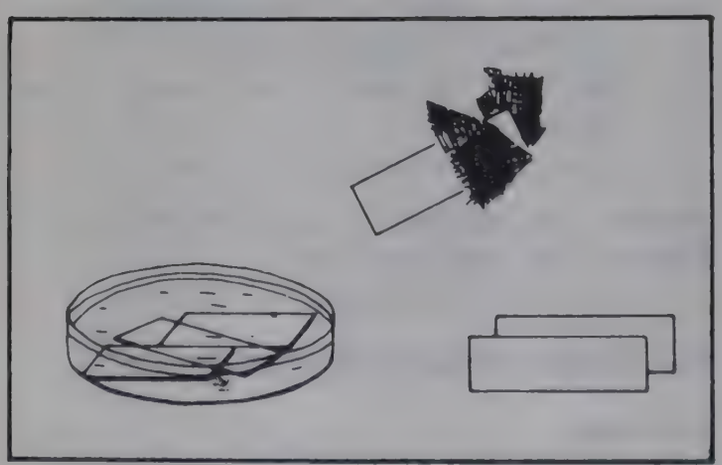
The actual size of the loop is shown in the figure.



If a wire loop is not available, you can use a clean broomstick.

– Glass slides: New if possible and unscratched.

Clean with an ethanol-ether mixture and wipe with gauze.

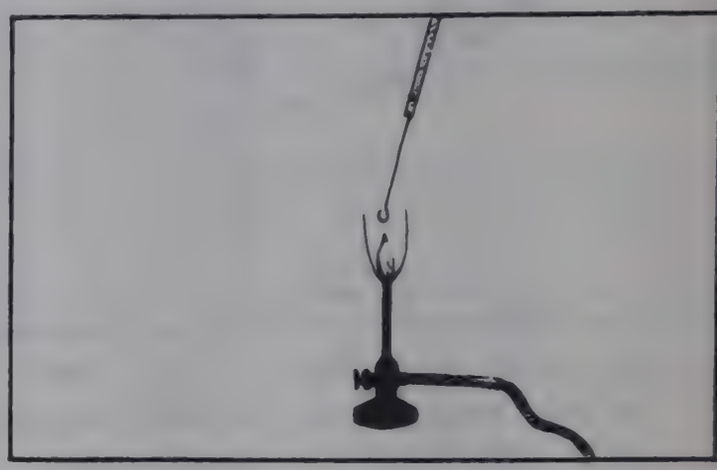


– Bunsen burner or spirit lamp
– Timer
– Filter paper
– Cotton wool plug on metal wire for flaming
– Matches
– Reagents (See Appendix 10.1)

Preparation of smear

(1) Enter the patient's number on the slide.

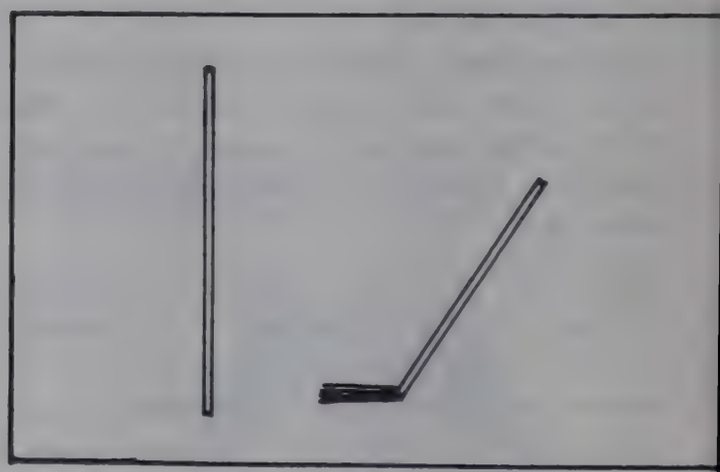
(2) Flame the loop until it is red-hot:
– hold the loop just above the blue part o the flame.
– hold the loop as nearly vertical as possible.



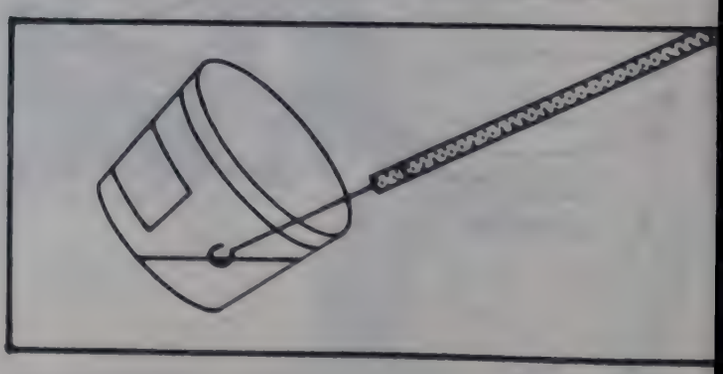
Allow the loop to cool (count to 20).

Or

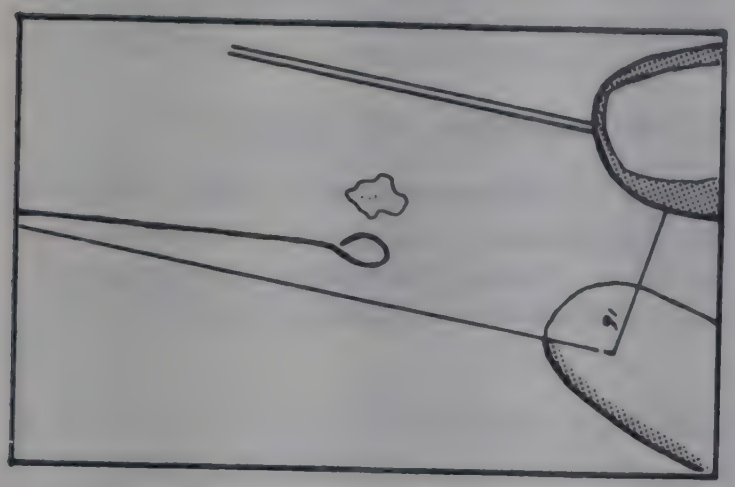
Crush and bend one end of the broomstick and use this end to make the smear.



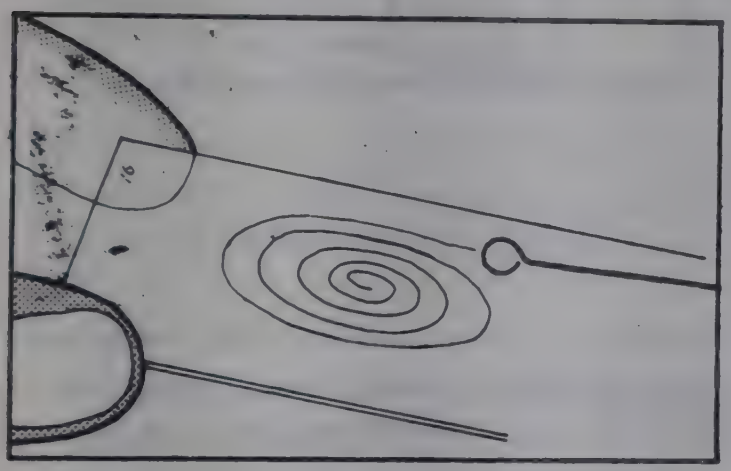
(3) Take a purulent portion of the sputum specimen by placing the loop or broomstick flat on the surface of the material.



(4) Place the loop or broomstick on the slide and press slightly, flat and in the centre.



(5) Still holding it flat against the slide, move the loop or broomstick in an oval spiral, outwards from the centre.



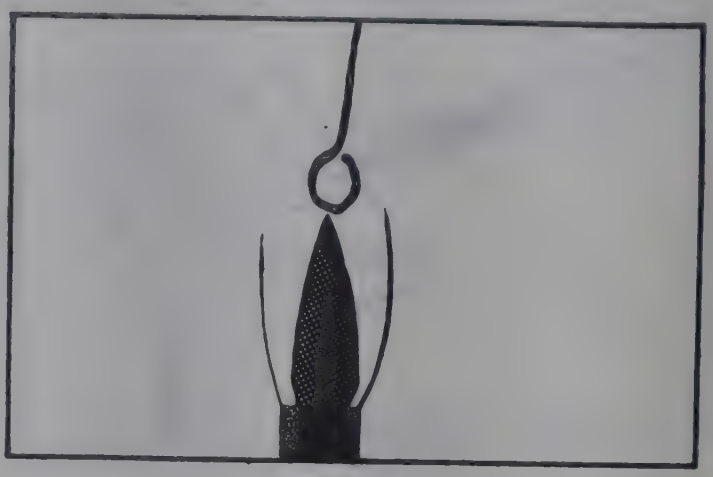
Leave a space between the specimen and each of the four sides of the slide:

Make the smears:
- as thin as possible
- covering as large an area as possible, tracing concentric circles well separated but not reaching the edges of the slide.

Let the slide dry completely in the air.

(6) When the smears are completed, plunge the wire loop into liquid disinfectant and shake to remove any sputum. Then bring the loop near the flame, wait until it is dry, then pass it through the flame. This prevents infected sputum from being sprayed into the air on exposure to the flame.

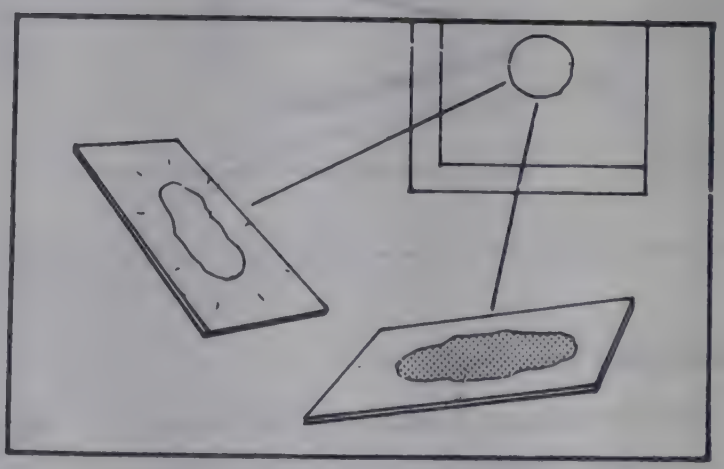
Flame the loop until it is red-hot to destroy any bacteria.



If a broomstick is used, it should be burned in the centre of the flame.

Note: Unmarked smears are sometimes received in the laboratory from outside sources. To find out on which side of an unmarked slide the smear has been made:

- Turn the slide so that it reflects the light from the window.
- The side without the smear will shine.
- The side with the smear will not reflect the light.

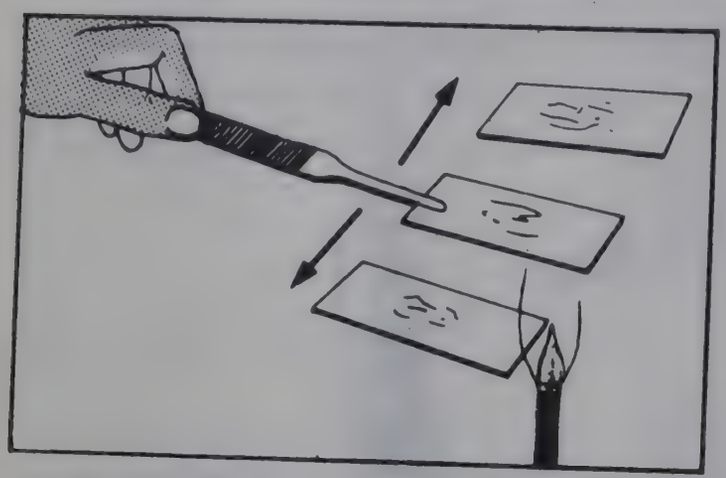


Fixation

(1) Dry the smears in the air.

(2) Check that the smear is completely air-dried.

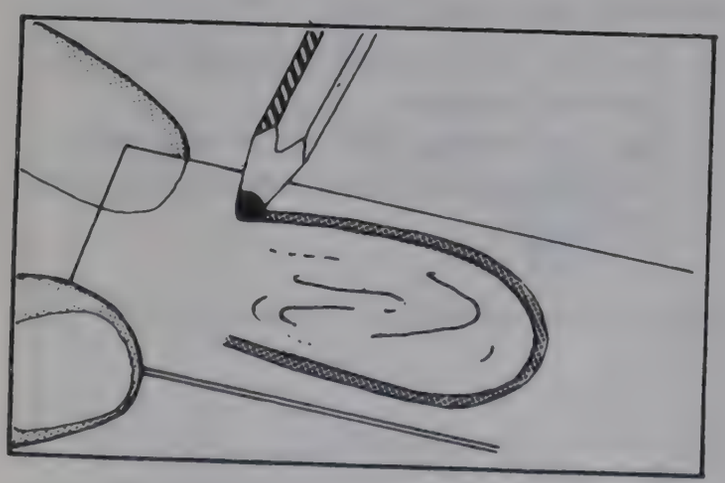
(3) Pass the slide three times through the flame of a Bunsen burner or spirit lamp with the smear uppermost.



Do not heat the slide too much or the smear will char.

(4) Allow to cool before staining.

(5) It is useful to draw a circle around the smear with a grease pencil, so that it can be seen more easily.

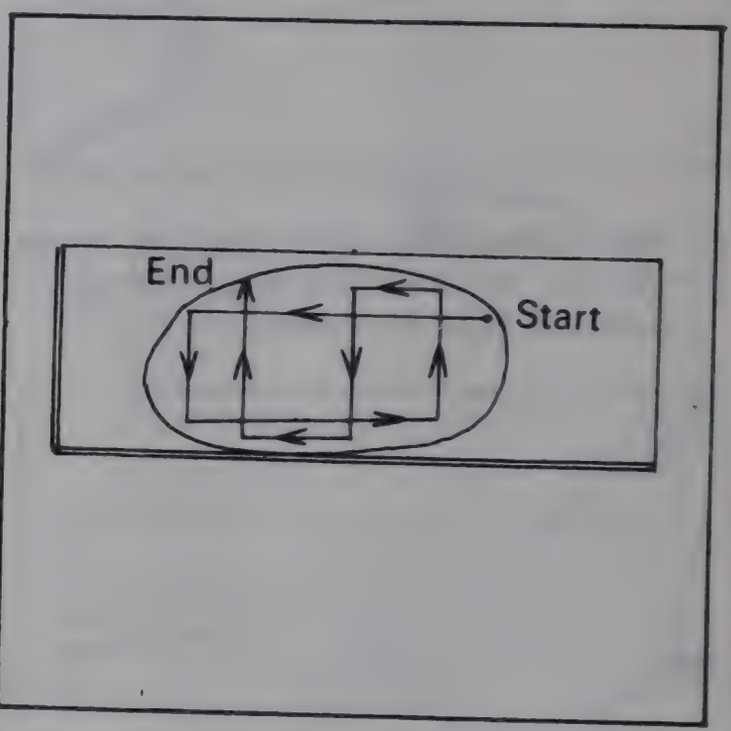


Staining

Stain the slides by Ziehl-Neelsen method (See Appendix 10.1).

5. Examination of Slides

Make a complete examination of the slide using the oil-immersion objective (maximum illumination), proceeding as shown in the diagram.



Before declaring a smear to be negative, examine the slide continuously for 5 minutes.

Recording of results

Number of acid-fast bacilli (AFB) found	*Report*
No bacilli found in 5 minutes examination	: Negative (Neg)
1–10 bacilli in 5 minutes examination	: State actual number found
More than 10 bacilli	: +
Masses (plenty of bacilli in several fields)	: ++

Appendix 10.1: Ziehl-Neelsen Staining

Principle

The tubercle bacilli (Mycobacterium tuberculosis) are acid-fast, i.e. once they are stained red by carbol fuchsin they cannot be decolorized by acid or by ethanol.

Carbol fuchsin – stains all the organisms in the sputum red.
Acid and ethanol – decolorize all organisms and cellular elements except acid-fast bacilli.
Methylene blue – stains blue all the organisms and elements decolorized by the acid and ethanol, but the acid-fast bacilli remain red.

Note: Another pathogenic acid-fast bacillus is the leprosy bacillus (Mycobacterium leprae).

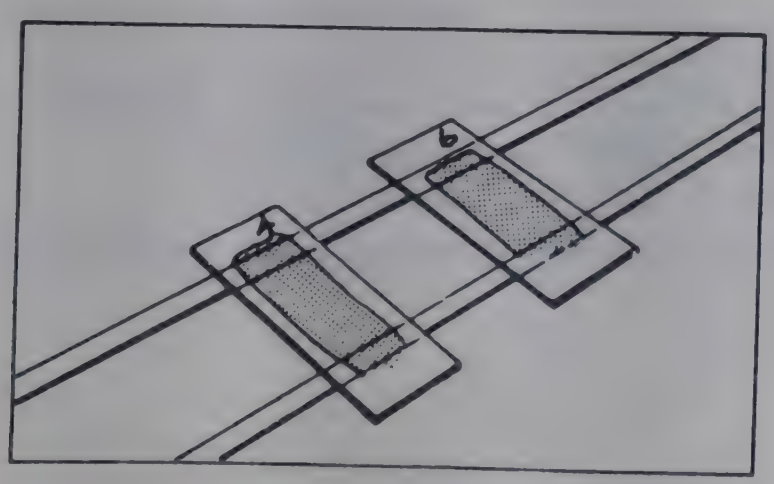
Reagents

- Carbol fuchsin for Ziehl-Neelsen stain (Reagent No. 7A.)
  This should preferably be filtered before use. Fresh stain should be prepared every three months.
- 25% Sulphuric acid*
- 90% Alcohol *or* methylated spirit
- Aqueous Methylene blue (Reagent No. 21A)
- Methylated spirit (for burning)
- Wash bottle of distilled water
- Tap water

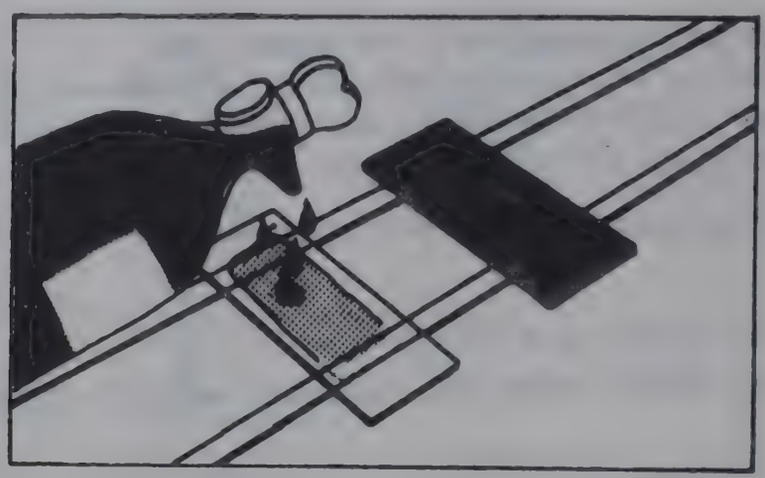
* Sulphuric acid 250 ml; Distilled water 750 ml.
Add acid very carefully to water in a large boiling flask, constantly mixing the contents. The flask should be kept in a sink with water for cooling the contents. Store in a glass stoppered bottle.

Technique

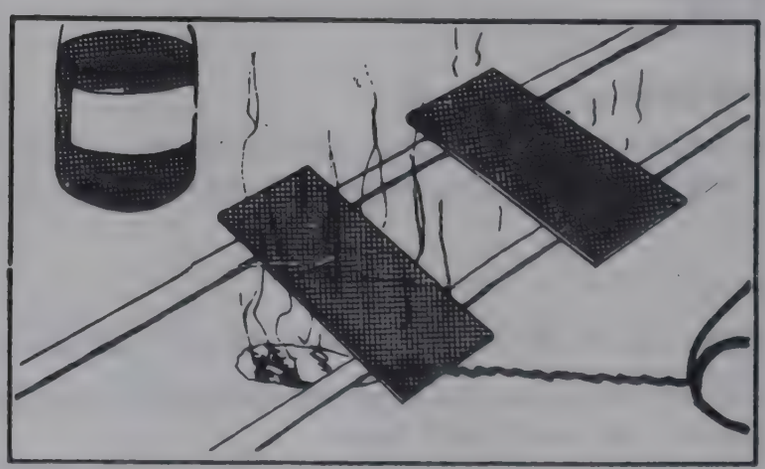
(1) After preparation the smears are fixed (as described in Chapter 10) and allowed to cool.
(2) Place the numbered slides on two glass rods over the staining tray with the smears facing upwards. The slides should not touch each other.



(3) *Carbol fuchsin staining* – 5 minutes with heat:
Cover the smear with a piece of cut filter paper.
Pour carbol fuchsin from a drop bottle on to the filter paper till it is completely covered.

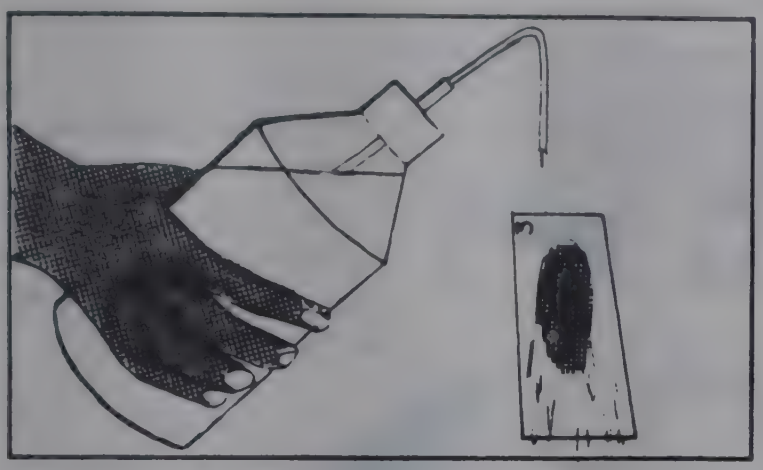


Dip the cotton wool swab in the methylated spirit, ignite and pass slowly under the slide to heat it.

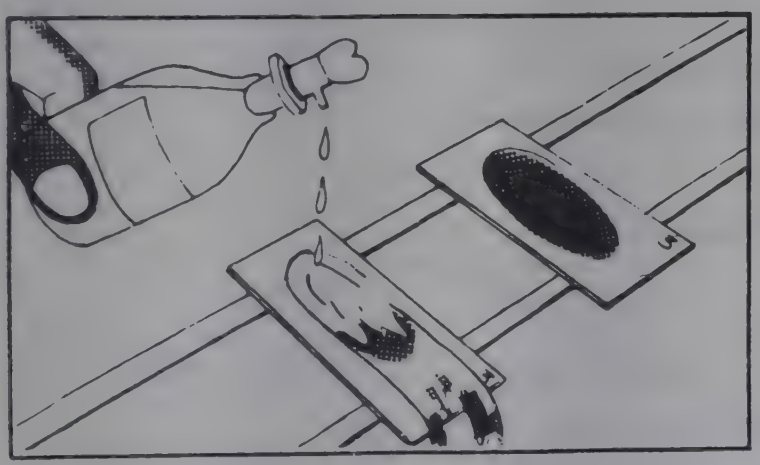


As soon as steam begins to rise, set the timer at 5 minutes.
After 2½ minutes heat it once again until steam is seen, but do not let it boil.
At the end of another 2½ minutes remove the filter paper.
The stain thus stays on the slide for 5 minutes.
If necessary add more stain during the 5 minutes so that the filter paper does not dry.

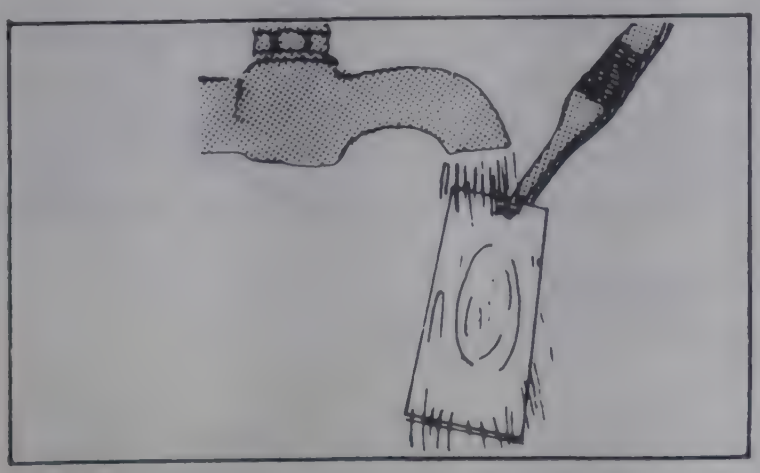
(4) *Wash with distilled water:*
Cool the slide.
Drain off the stain.
Wash the slide gently with water until the water that runs off is colourless



(5) *Decolorization with acid and ethanol:*
Cover the slide with 25% sulphuric acid.

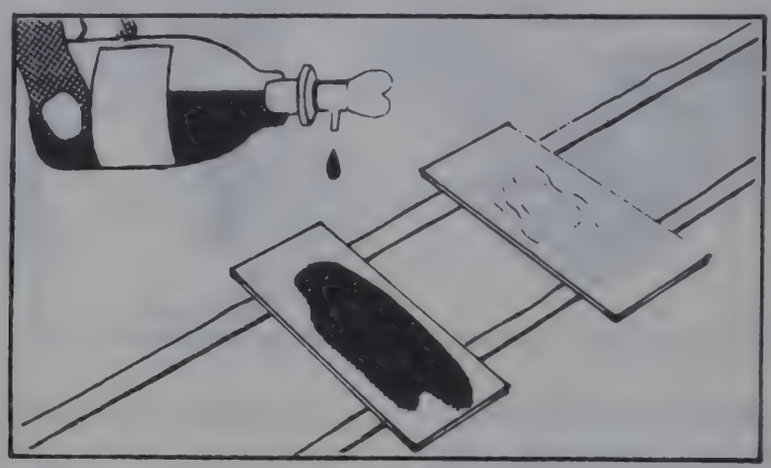


Leave for 2½ minutes to remove the colour of carbol fuchsin.
Wash the slide in ordinary tap water and drain.



Pour acid on the slide again and leave for 2½ minutes.
Wash with water and drain. The smear may now have a faint pink colour.
Pour 90% alcohol or methylated spirit on the smear and leave for 2 minutes.
Wash with water and drain.

(6) *Methylene blue counterstaining* – 10 seconds:
Cover the slide with the stain.

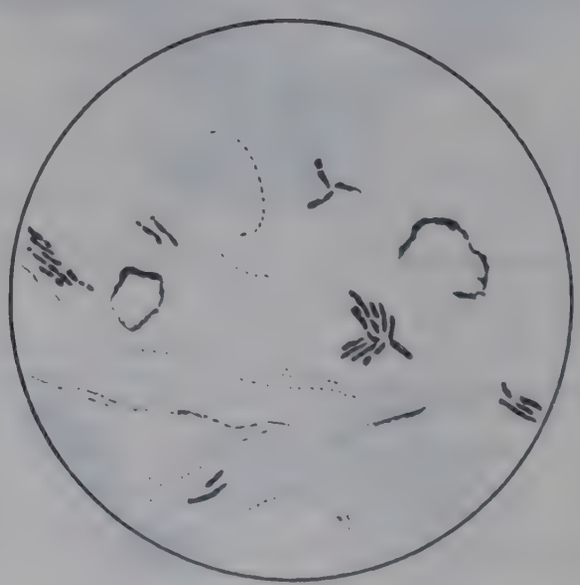


Leave for 10 seconds.
Wash well with tap water and drain.
Leave to dry in air on a slide rack.

What to look for

Use the oil-immersion objective.

Tubercle bacilli are:



- bright red on a blue background
- straight or slightly curved
- quite short (1–4 μ)
- often granular

arranged in groups of 3–10 bacilli close together, like bits of string, or forming letters or forked shapes. (They are often found near threads of fibrin.)

CHAPTER 11

Examination of Skin Smears and Nasal Smears

Leprosy is caused by a germ—the Mycobacterium leprae. Leprosy is of two main types—lepromatous and non-lepromatous. Leprosy bacilli are present in large numbers in lepromatous lesions, but are usually sparse or not seen at all in non-lepromatous lesions.

1. Slit Skin Smear

Principle

A lesion is incised superficially without causing bleeding. Serous material from the incision is spread on the slide, air-dried, fixed by heat, and examined under a microscope after staining by a modified Ziehl-Neelsen method. Mycobacterium leprae is acid-fast.

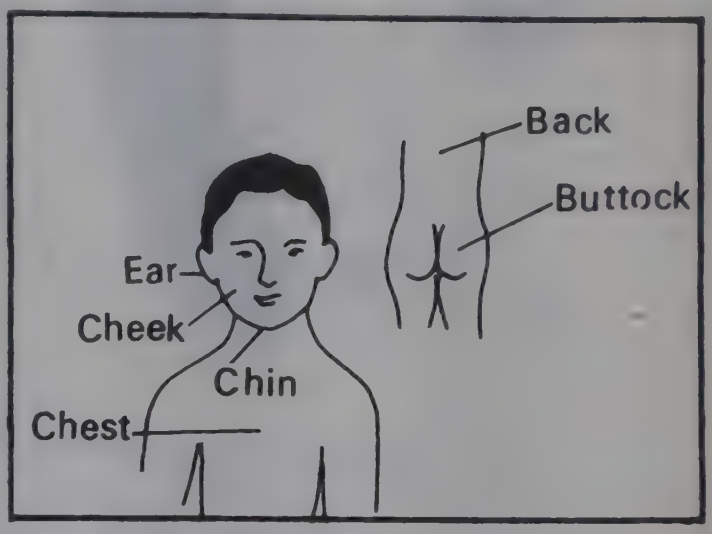
Materials

- Skin pencil
- Bard-Parker scalpel
- Spirit lamp
- Slides numbered with a glass marking pencil
- Gauze
- Cotton wool
- Ethanol

Selection of site

The site from which the smears have to be taken should usually be marked by the doctor with a skin pencil. However, in selecting the site, the following points should be remembered both by the doctor and by the laboratory technician.

(1) In cases where *no* patches are seen, the smears should be taken from the following sites:



i. Ear
ii. Cheek
iii. Chin
iv. Chest
v. Back
vi. Buttock.

(2) If patches are seen in other sites besides the six mentioned above, smears should also be taken from the patches. Where the number of patches is less than five, smears should be taken from *all* the patches in addition to the six sites mentioned.

(3) Smears should be taken from the edge of the skin patches where the disease is most active, and should also include a part of the normal skin on the edge.

Method

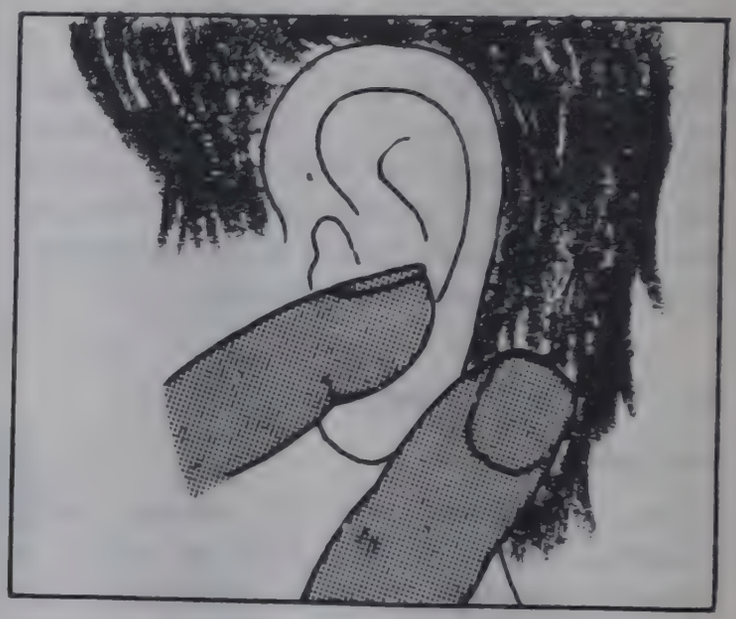
A. Taking a specimen from the ear

(1) Select the site from which the smear is to be taken.

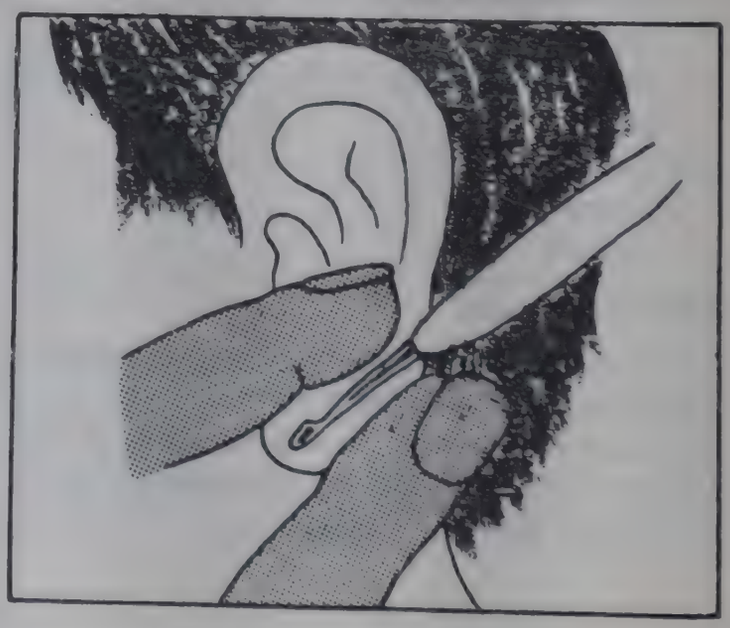
(2) Clean the area with a gauze swab moistened with ethanol.

(3) Flame the scalpel. Scalpels should be sterilized beforehand in small glass tubes plugged with non-absorbent cotton wool. Avoid iodine.

(4) Pinch the ear lobe hard between the finger and thumb to stop the flow of blood to the area.

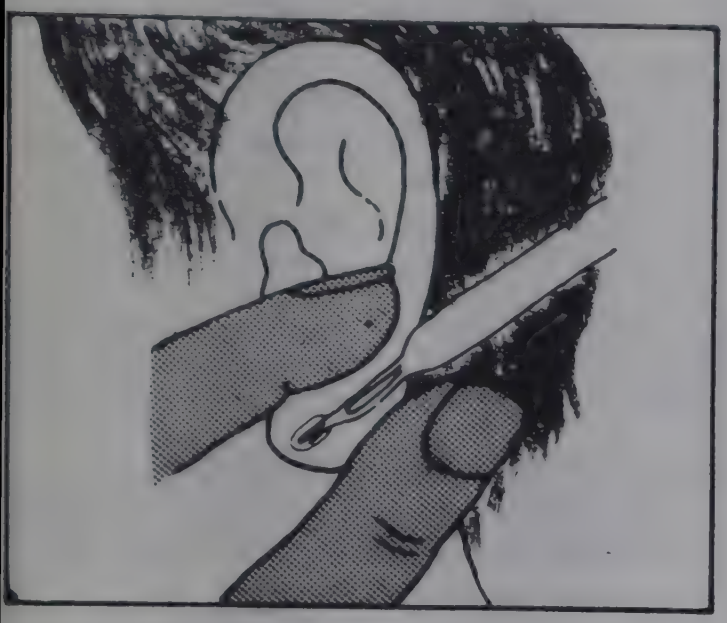


(5) Make a superficial incision lengthwise at the edge of the lesion, about 5 mm long and 2 to 3 mm deep. Do not draw blood.



If blood appears, wipe dry with dry sterile gauze and pinch the skin harder to stop the bleeding.

(6) Still squeezing with the fingers, scrape the bottom and edges of the incision with the point and blade of the scalpel.

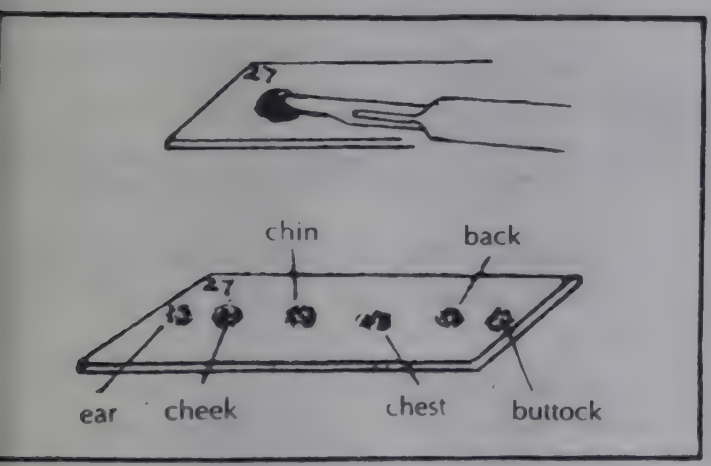


Collect on the scalpel the colourless or pinkish serous material from the lesion.

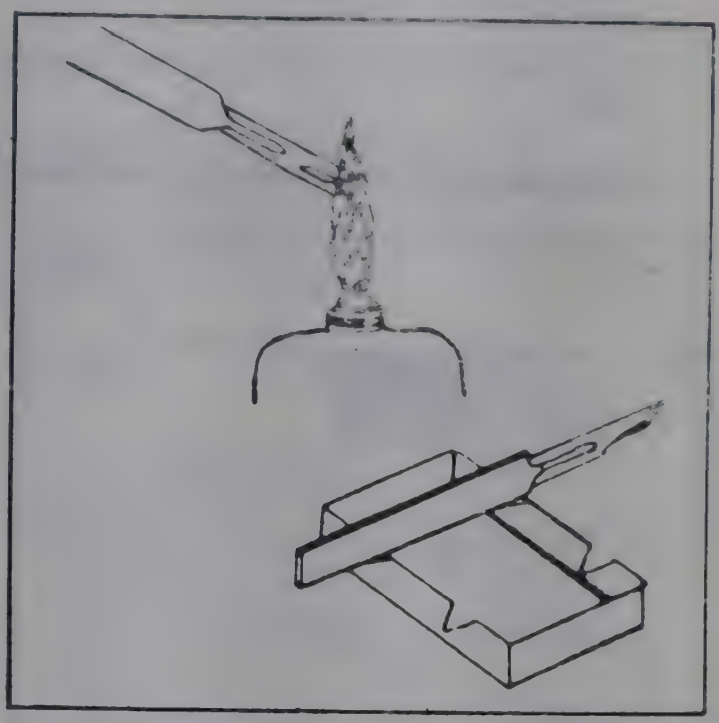
(7) With the flat of the blade spread the serous material evenly in a circular motion over an area 5 to 7 mm in diameter on one end of the slide.

Five to six smears from different sites from the same patient may be put on a single slide.

Mark the slide with the patient's number.



(8) Wipe the scalpel with cotton wool moistened with ethanol. Flame the scalpel in the spirit lamp for 2 seconds. Place the scalpel on a stand to cool before using it again for taking smears from other lesions in the *same* patient.



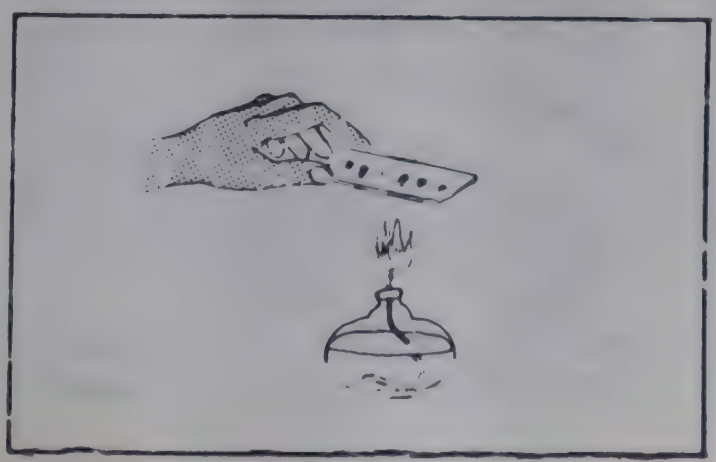
Do not heat the scalpel too long or it will get blunt. Do not let it get red hot.

(9) Clean the incision with ether or ethanol

Place a piece of dry sterile cotton wool over the cut. Apply tincture benzoin to seal the cut.

(10) Leave the slide to dry in a dust-free place.

When the smears are completely dry, fix the smears by passing the slide through the flame of a spirit lamp 2 to 3 times, heating the back of the slide slightly. Keep the surface carrying the smears uppermost.

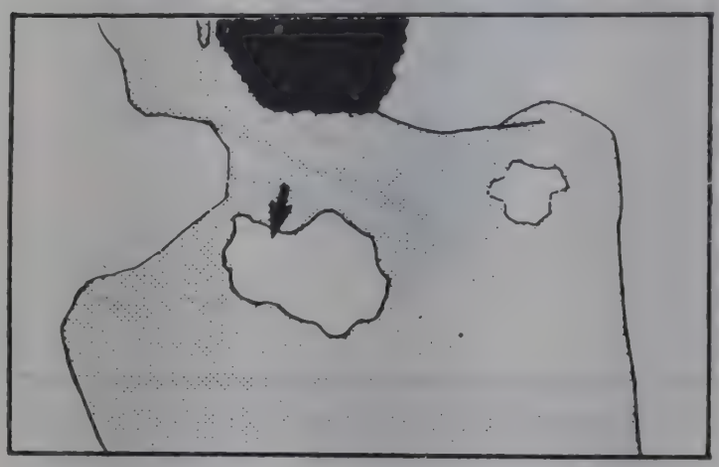


Do not let the slide char. Avoid too much heat which will result in cracking of the smear, and its being washed out during staining.

(11) Burn the soiled cotton wool and gauze swabs.

B. Taking specimens from the body and face

(1) Select the site from which the smear is to be taken.



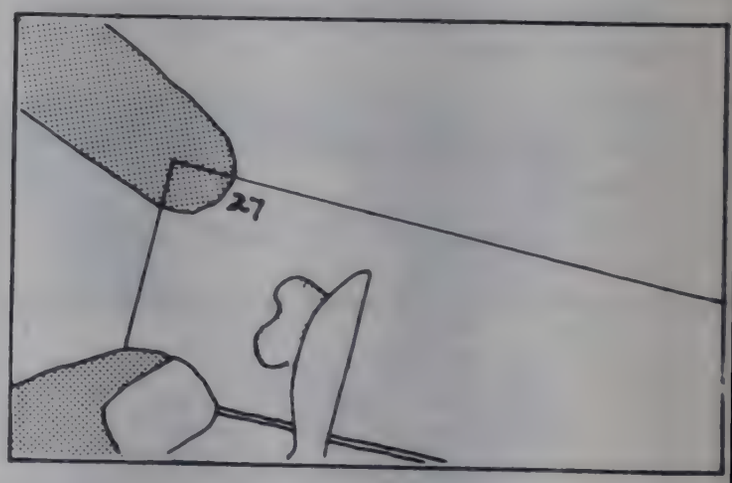
(2) Clean the area with gauze dipped in ethanol.

Pinch the site hard between finger and thumb.

(3) Continue to hold firmly. With the tip of a scalpel make an incision 5 mm long and 2 to 3 mm deep.

(4) Scrape the bottom and edges of the incision with the tip of the scalpel. Collect a quantity of pulp and serous material. Continue to squeeze to prevent bleeding.

(5) With the scalpel spread the specimen in a circular motion over an area 5 to 7 mm in diameter on a glass slide numbered with a glass marking pencil.



5 to 6 specimens from different lesions from the same patient can be put on the same slide.

Wipe the scalpel with cotton wool moistened with ethanol. Flame the scalpel in a spirit lamp for 2 seconds. Place it on a stand to cool before using it for taking smears from other lesions in the *same* patient.

(6) Dry and fix the smears as already described under Section A.

(7) Clean the incision with ether or ethanol and seal the cut with tincture benzoin.

(8) Collect the soiled cotton wool and gauze swabs in a covered container and burn them later. Boil the container or soak it in phenol.
Care in handling infected material will protect you from infection.

Note: After taking all the skin smears from a patient, wash the scalpel well with detergent and water. Dry and place the scalpel in a suitable metal container for autoclaving. It is most important to sterilize the scalpel before using it on another patient. This will avoid cross infection.

2. Nasal Smear

Specimens are best prepared from an early morning 'nose blow'.

Materials

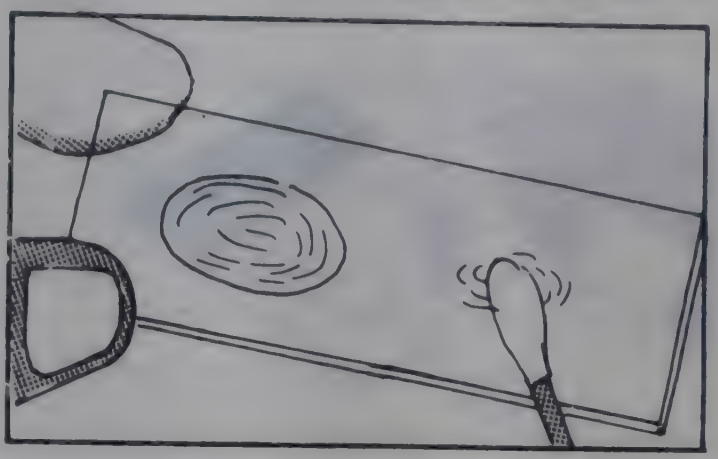
- Sheet of blotting paper
- Cotton wool swab on stick
- Slides numbered with a glass marking pencil
- Spirit lamp

Method

(1) The patient blows his nose thoroughly into a small clean dry sheet of blotting paper.

(2) With a small cotton wool swab, transfer some of the opaque material from the blotting paper to a labelled microscope slide.

(3) Spread the material as evenly as possible on the slide. Two or more smears of the same material may be made on one slide.



(4) Leave the smears to dry.

(5) When completely dry, fix by heat as described under slit skin smears.

(6) Burn the soiled blotting paper and swabs.

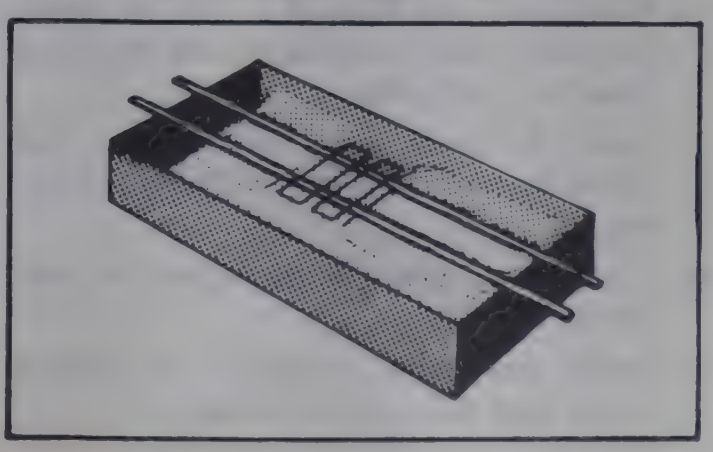
3. Staining the Smears (Modified Ziehl-Neelsen method)

Materials

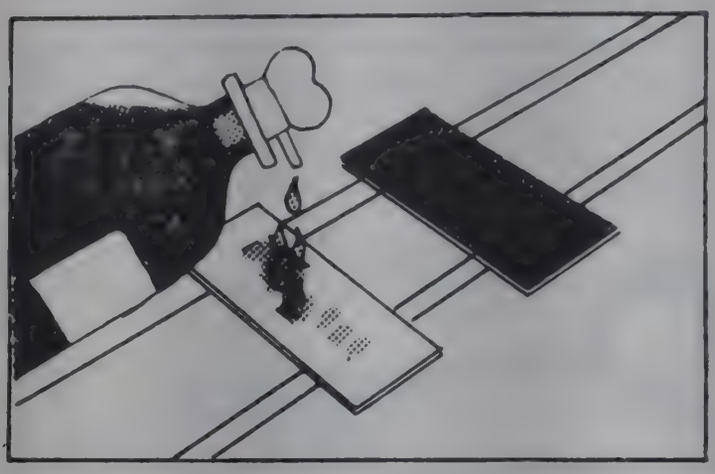
- 2 glass rods over sink or staining tray
- Beaker
- Carbol Fuchsin for modified Ziehl-Neelsen stain (Reagent No. 7B)
- Acid-Ethanol (Reagent No.2)
- Methylene Blue counterstain (Reagent No. 21B)

Method

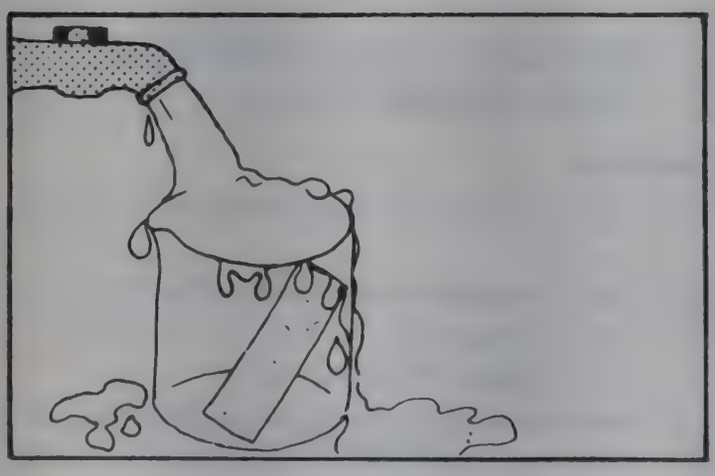
(1) Place the slides with smears already fixed on the staining trough or glass rods.



(2) Flood each slide with *freshly filtered* carbol fuchsin and allow t stand for 20 minutes.



(3) Wash the slides gently, preferably in running water (in a beaker with the smear away from the stream of water)



They may be allowed to stand in the beaker until decolorization.

(4) Decolorize one slide at a time by gently streaming with acid-ethanol until this flows clear and colourless (5 minutes).

(5) Wash the slides again in tap water and allow them to stand wet until counterstaining.

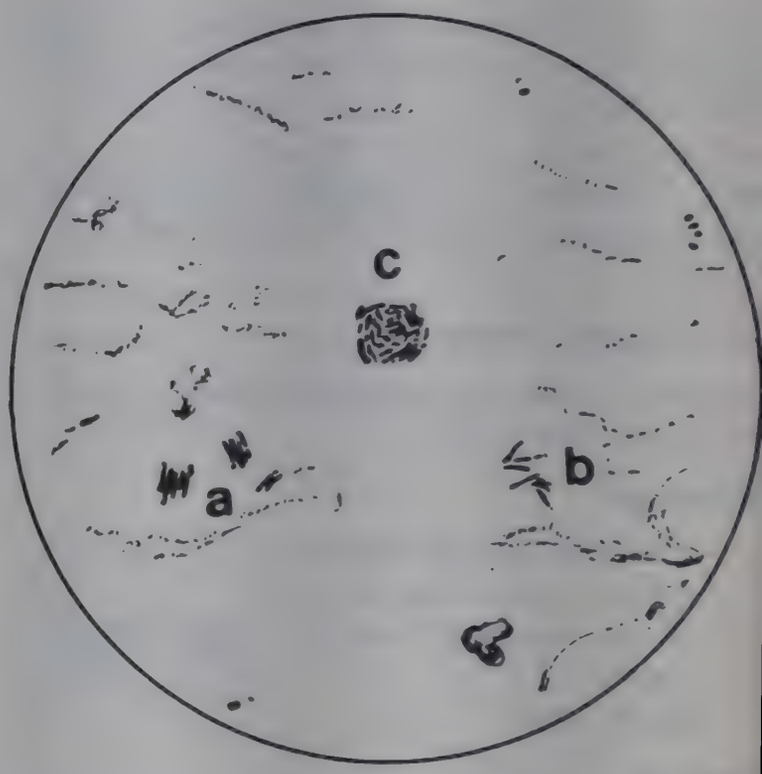
(6) Counterstain with methylene blue solution for 30 seconds.

(7) Wash thoroughly, preferably in running water and then let the smear dry.

4. Examination of Smear

Examine the smear first with the × 5 eyepiece and the × 10 objective. Select a good field, i.e. one that shows plenty of tissue cells and is neither too thick nor too thin. Examine with the × 100 objective. Examine at least 50 fields.

The Mycobacterium leprae is similar in appearance to the tubercle bacillus. Like the latter, it is acid-fast and stains red on a blue background with the modified Ziehl-Neelsen technique.



Size : 1–8 μ

Shape : longish rods straight or slightly curved with rounded ends

Granulation : often granular, with bright red granules separated by colourless spaces

Arrangement : (a) either in groups of 2–5 arranged in parallel
(b) or in larger groups or clusters
(c) or in large numbers in circular masses called 'globi'.

After the smear has been examined, place the slide in a covered enamel bowl containing a

solution of 5% phenol or similar disinfectant. At the end of the day boil the slides in the bowl for 10 minutes. When cool, follow the procedure for cleaning dirty slides described in Chapter 1, Section 3(4)B, p. 10.

Cross out the number marked on the slide and use the other side of the slide. Each slide can be used twice for collecting skin or nasal smears *provided* the slide is not scratched. After that the slide can be used for microscopic examination of urine or stools.

Note: To avoid false positive reports it is advisable to break and discard all AFB positive slides. Before discarding the slides boil them in phenol solution.

5. Recording the Result

(1) State the site from which the smear was taken, e.g. ear nodule, chin, patch on back, nasal blow, etc.

(2) State whether or not AFB are seen. Specify whether they are seen in globi.

(3) Indicate the degree of positivity:

Slight	+	Bacilli not found in every field but only in an occasional field and there are not more than 2 or 3 bacilli in a field, perhaps with one or two small globi in 50 fields.
Moderate	++	Bacilli found in every field, but not more than about 10 in each field, with a few globi here and there.
Heavy	+++	Numerous bacilli and globi found in every field.
Massive	++++	Innumerable bacilli and large number of globi found in every field.

CHAPTER 12

Examination of Semen

Semen is the fluid discharged during ejaculation. It consists of spermatozoa from the male genital organs, viz. the testes, and secretions from the seminal vesicles and prostate gland.

Examination of semen is done in:

(a) Infertile couples – to find out whether the man is able to produce normal spermatozoa;

(b) Cases following vasectomy – to check that no spermatozoa are present in the semen.

1. Collection of Semen

(1) Prior to collection the man should refrain from ejaculation for at least 2 days but not more than 5 days.

(2) The specimen may be collected by masturbation or coitus interruptus.

(3) The specimen should be collected in a clean dry container and should be brought to the laboratory preferably within 15 minutes and at least within 1 hour of ejaculation.

(4) Note the date, time of collection, and time of receiving the specimen.

2. Macroscopic Examination

(1) Volume

Measure the semen collected in a graduated cylinder. Record the amount. (Normal volume: 2–5 ml)

(2) Colour

Normal semen is opaque, white, or greyish in colour.

It should not contain blood, pus or mucus.

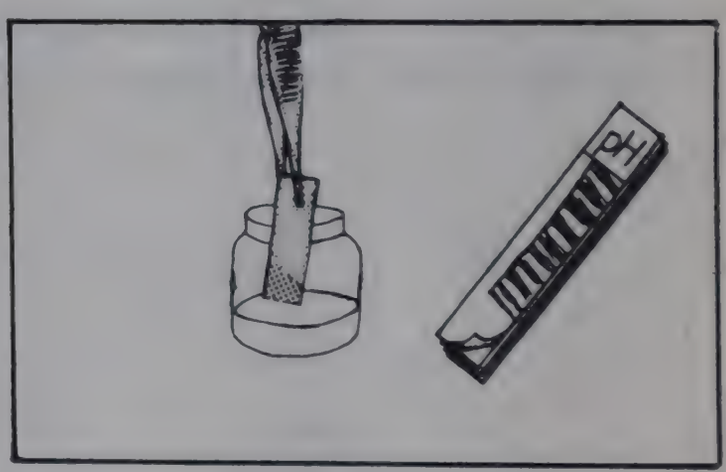
(3) Consistency and liquefaction

Observe the consistency of the semen at the time of receiving the sample and note the time taken for the specimen to liquefy.

Freshly ejaculated semen is viscid and coagulates promptly. Normally it liquefies within 20 to 60 minutes.

(4) pH

Dip a strip of pH indicator paper (range 6.0–8.0) in the semen and read off the pH unit given for the colour most closely matching the test paper.



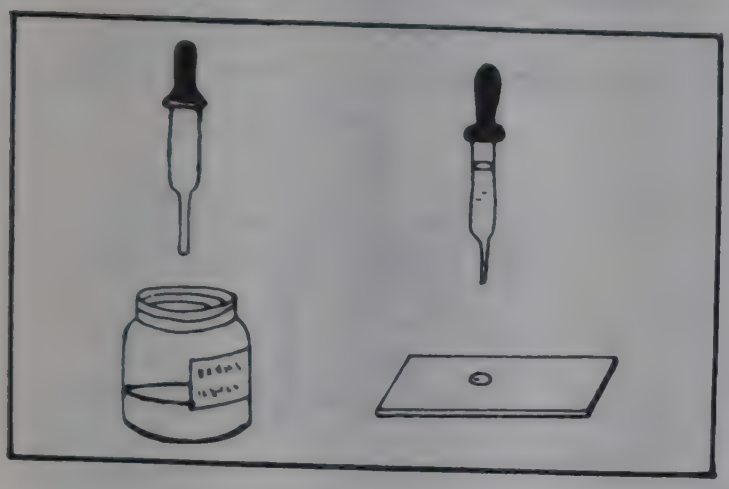
The normal pH ranges from 7 to 7.7.

3. Microscopic Examination

(1) Sperm motility

This should be tested *as early as possible* after ejaculation. The test should be carried out at room temperature.

i. Place a drop of semen on a clean slide.



ii. Cover the drop with a coverslip.

iii. Rim the edges of the coverslip with vaseline to prevent drying.

iv. Examine the slide with the × 10 objective. If spermatozoa are seen, examine the slide with the × 40 objective.

Reduce the illumination by adjusting the iris diaphragm.

v. Count the number of actively motile, sluggishly motile and non-motile sperm among 100 spermatozoa.

Normally about 80% of spermatozoa are actively motile and about 20% are sluggish or not moving at all.

vi. Observe the slide and count the motile spermatozoa again after 3 hours, after 6 hours and, if convenient, after 12 hours.

Normally up to 3 hours the spermatozoa remain motile. After this the motility progressively decreases. By 12 hours 80% are non-motile.

(2) Sperm count

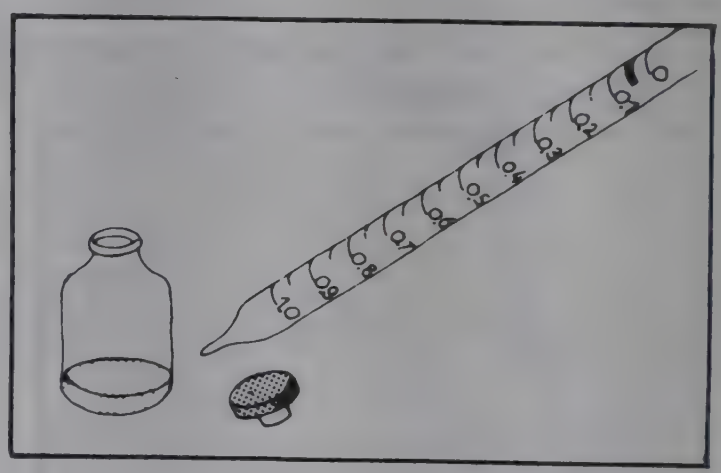
Materials

– Improved Neubauer counting chamber with special coverglass
– Formalin-bicarbonate diluting fluid* (Reagent No. 27)
– WBC pipette
– 1 ml graduated pipette
– Pasteur pipette
– Microscope

* Formalin immobilizes the spermatozoa so that they can be counted.

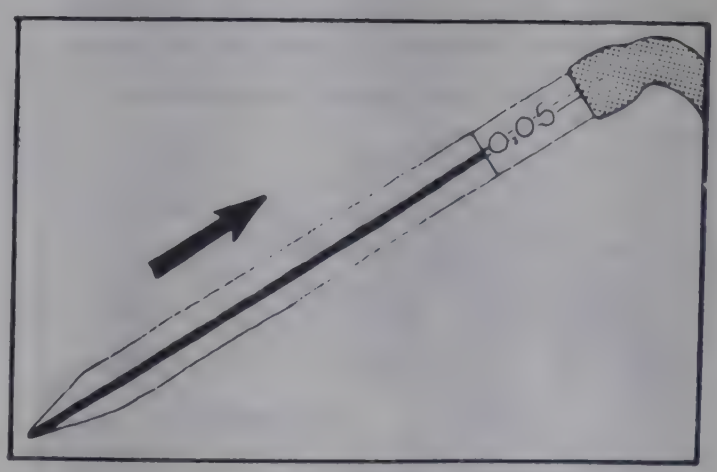
Method

i. Pipette 0.95 ml of diluting fluid into a small bottle using the 1 ml graduated pipette.

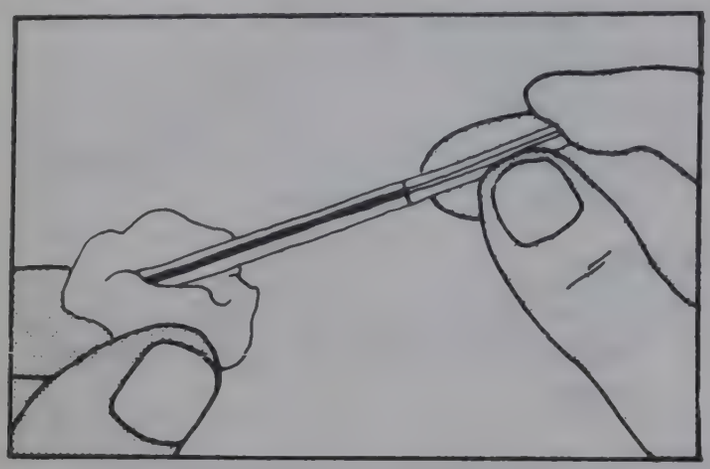


ii. Mix semen specimen thoroughly by shaking gently.

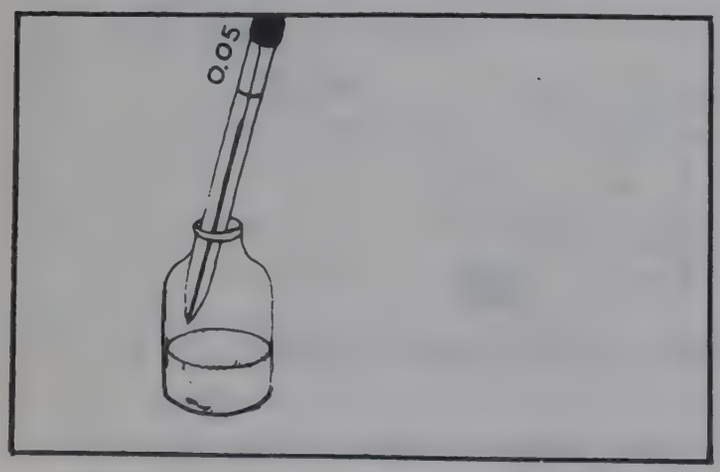
iii. With the WBC pipette draw semen up to the 0.05 ml mark.



iv. Wipe the outside of the pipette with filter paper. Check that the semen is still on the mark.

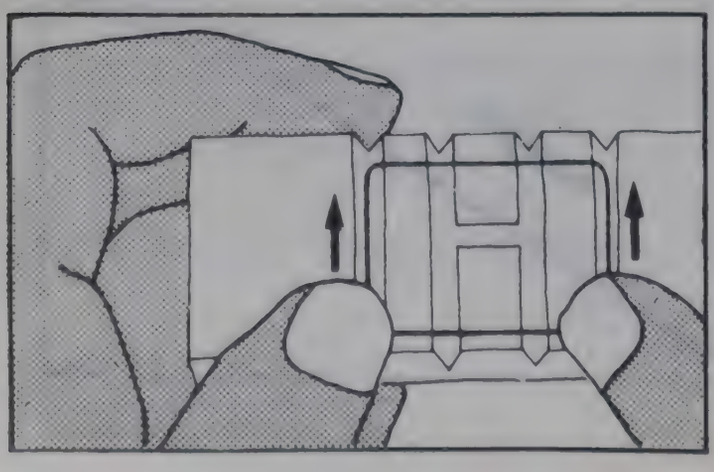


v. Blow the semen into the bottle containing 0.95 ml of diluting fluid (1 : 20 dilution).

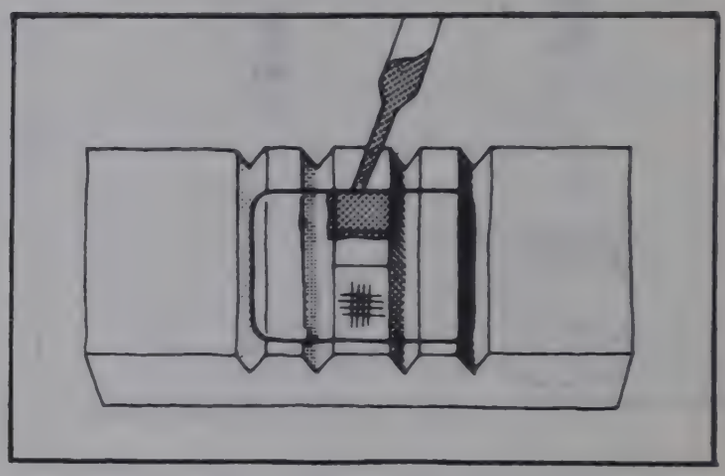


vi. Rinse the pipette by drawing in and blowing out the fluid three times. Mix well the diluted semen.

vii. Attach the coverglass to the Neubauer chamber, pressing it carefully into place.



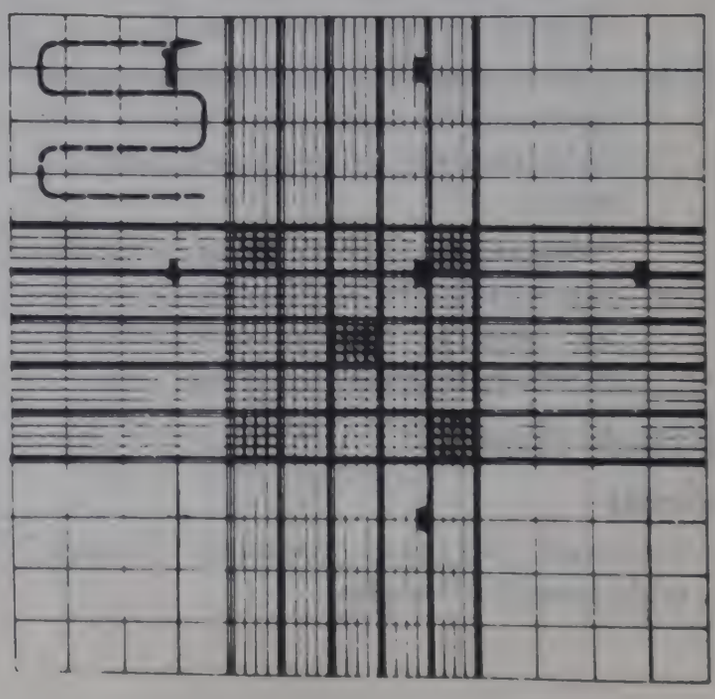
viii. Using a Pasteur pipette load the counting chamber with diluted seminal fluid.

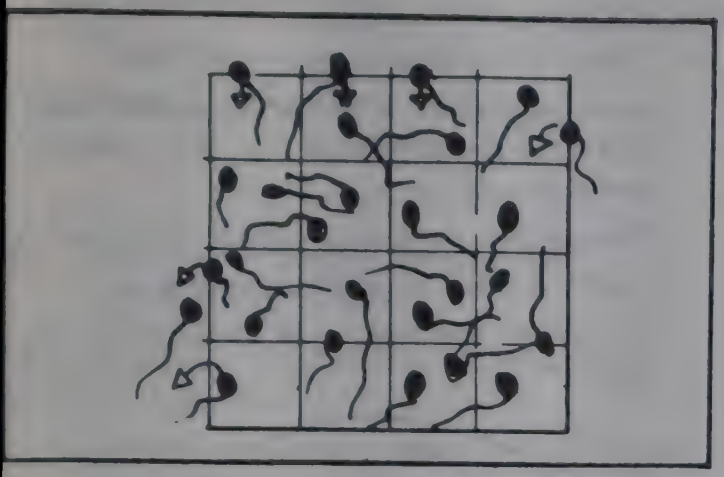


ix. Leave the chamber on the bench for 3 minutes.

This will allow the sperms to settle down.

x. Count the number of spermatozoa in one large corner square, i.e. an area of 1 mm^2.





Use the × 10 objective and the × 6 eyepiece.

While counting, consider only complete spermatozoa with heads and tails.

Calculate the number of spermatozoa in 1 ml of semen:

Multiply the number of spermatozoa counted in one large corner square (i.e. 1 square mm) by 200,000.

Report the number as : Spermatozoa in millions per millilitre.

Example:

Number of sperms counted = 380
Sperm count = 380 × 200,000
=76,000,000
i.e. 76 millions/ml

Explanation of calculation:

The square in which sperms are counted has an area of 1 mm^2.

The chamber depth is 0.1 mm, therefore the volume in which sperms are counted is × 0.1 = 0.1 mm^3.

Thus, multiplication by 10 will give the number of sperms in 1 mm^3 of diluted semen.

Since the dilution is 1 in 20, multiplication by 20 will give the number of sperms in 1 mm^3 of undiluted semen.

1 ml = 1,000 mm^3, therefore multiplication by 1,000 will give the number of sperms in 1 ml of undiluted semen.

Thus, if the number of sperms counted is 380,
Sperms per ml = 380 ×10 ×·20 × 1,000
= 380 × 200,000
= 76,000,000
i.e. 76 millions per ml

Normal sperm count: 60–150 millions/ml

(3) Sperm morphology

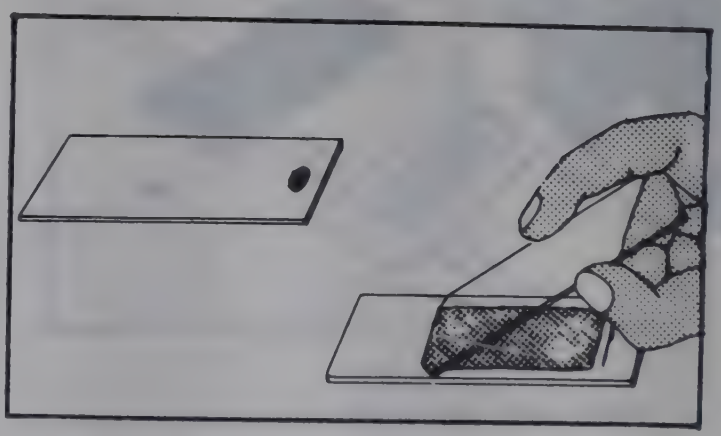
Materials

- 0.25% of aqueous solution of basic fuchsin stain (Basic fuchsin 0.25 g; Distilled water 100 ml)
- Slide and coverslip
- Microscope

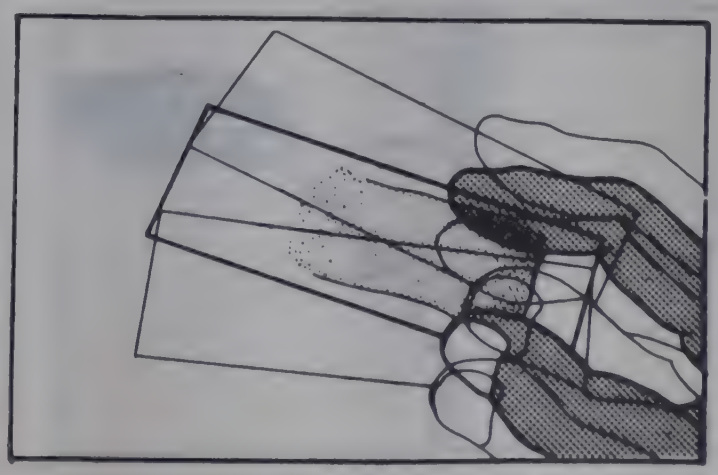
Method

i. Allow spontaneous liquefaction of semen to be completed (½ – 1 hour).

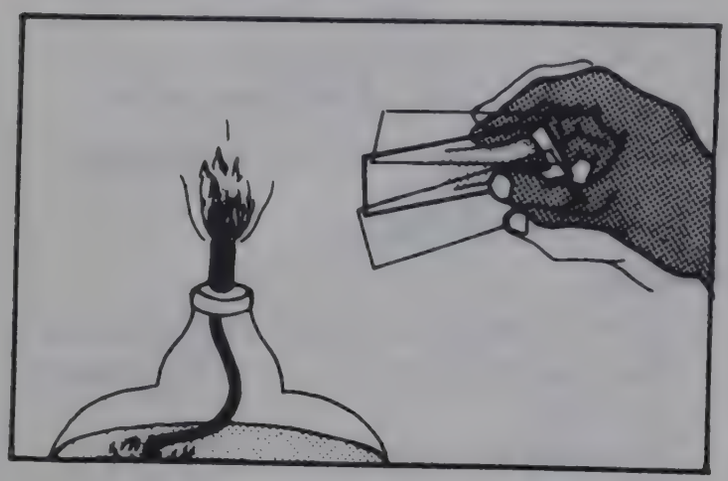
ii. Prepare a smear from the semen (as for thin blood smear, Ch. 9, Section 7(1), page 137.



iii. Dry the smear in air.

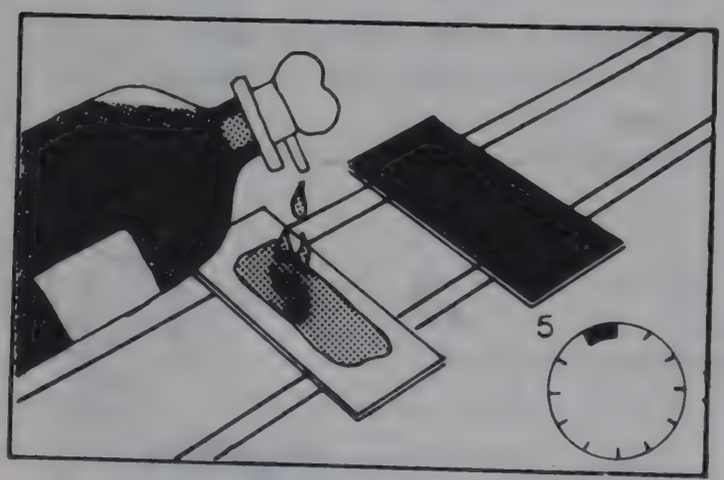


iv. Fix the smear by passing it quickly over a flame.

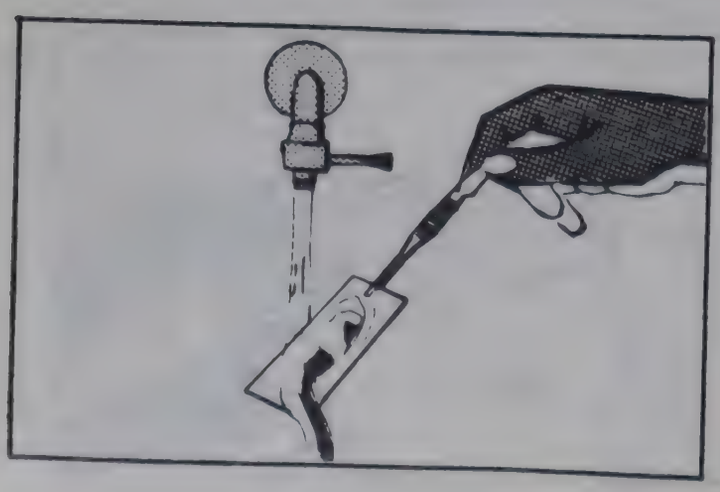


v. Cool the slide to room temperature.

vi. Cover the slide with basic fuchsin stain for 5 minutes.



vii. Wash with tap water. Blot dry.



viii. Examine under the oil immersion lens.

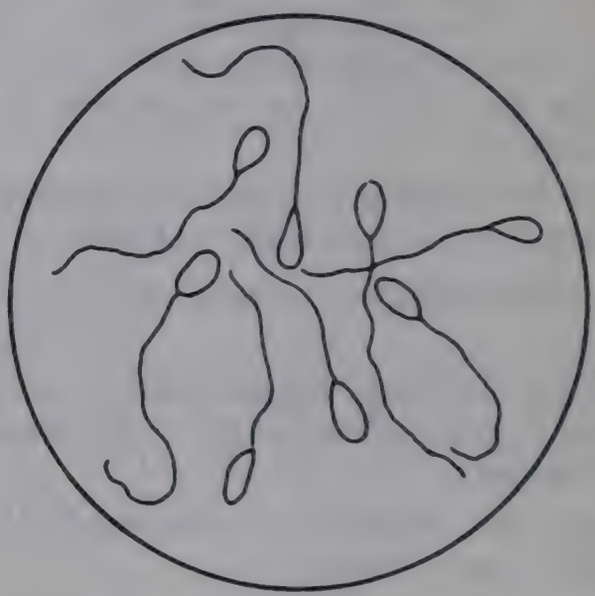
ix. Count *all* spermatozoa in the field.

Count abnormal forms seen among these sperms, i.e. defects in the head, middle piece or tail.

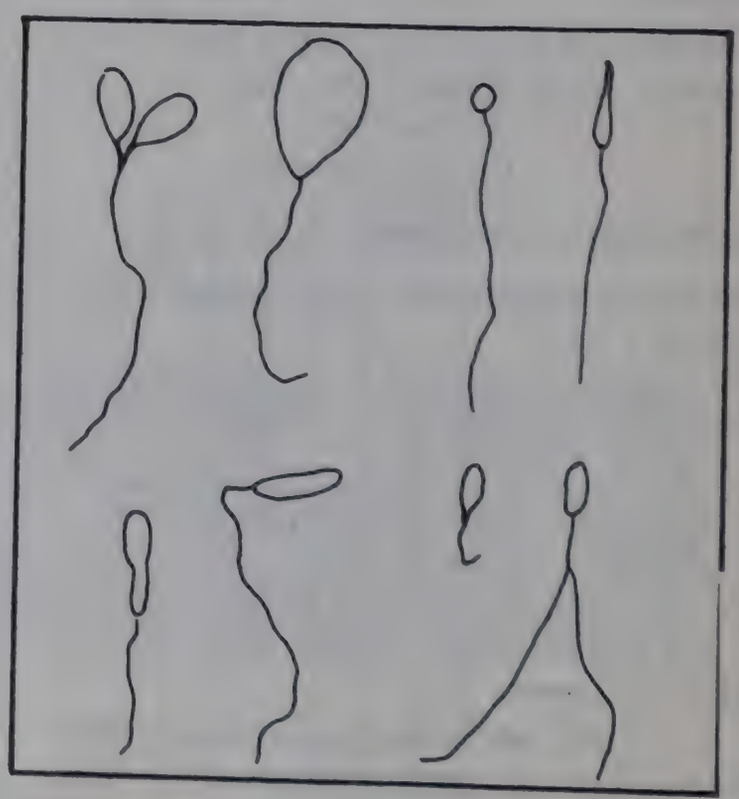
Change the field and repeat the count.

Count a total of 100 spermatozoa.

Record the percentage of abnormal forms seen.



Normal spermatozoa



Abnormal spermatozoa

Abnormal forms include:

Head	: pinheads; large heads; double heads;
Middle piece	: absent; bifurcated; swollen;
Tail	: double; curled; rudimentary; absent;

4. Semen Examination in Post-Vasectomy Cases

Usually semen examination is done 3 months after the operation.

For operated cases a period of abstinence before examination is not necessary.

After liquefaction, one drop of ejaculate is placed on a slide, covered with a coverslip and observed under the × 10 objective of the microscope. Not a single motile sperm not even dead sperm should be present in the semen. Only then can the ejaculate be said to be azoospermic'. If any spermatozoa are seen, call the man for a re-examination after 15 to 30 days. Re-examinations are done until the ejaculate is free of sperms. Until then the man must be advised to use nirodh.

CHAPTER 13

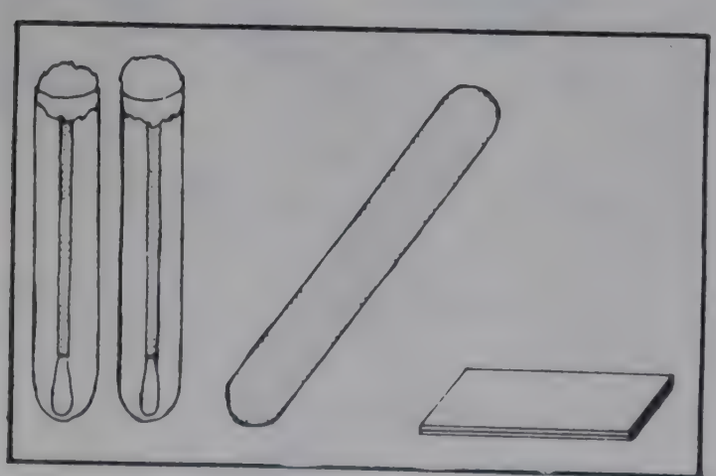
Examination of Throat Swab

It is rarely possible to diagnose a disease in the laboratory on the basis of the identification of the organisms found by direct bacteriological examination of a specimen. However, direct microscopical examination of a stained smear prepared from a throat specimen sometimes gives an indication of the organism causing an infection. It can help the physician to establish a diagnosis when taken together with the symptoms shown by the patient. Bacterial culture is needed to establish the identity of the organism with certainty.

Two organisms responsible for throat infections include:

- Diphtheria bacilli (Corynebacterium diphtheriae)
- Streptococci

1. Materials



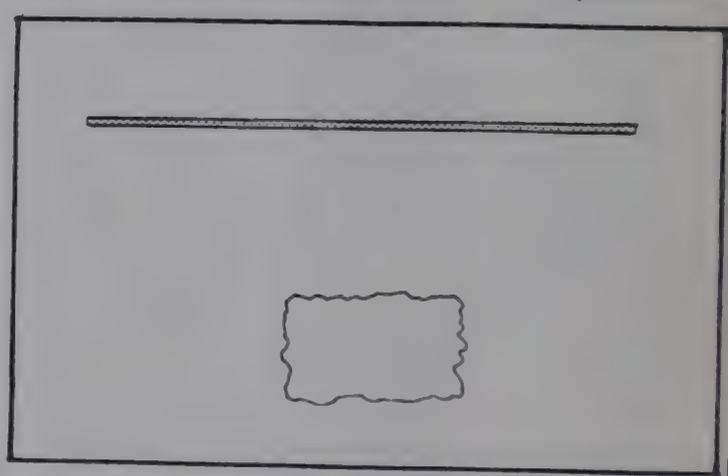
- Sterile cotton wool swabs in test tubes for the collection of specimens*
- Tongue depressor or spoon
- Slides
- Reagents for Gram staining (See Appendix 7.1)

*** Preparation of swabs**

If possible swabs prepared at the district laboratory should be made available to the PHC laboratory; if this is not possible, the following technique might be used.

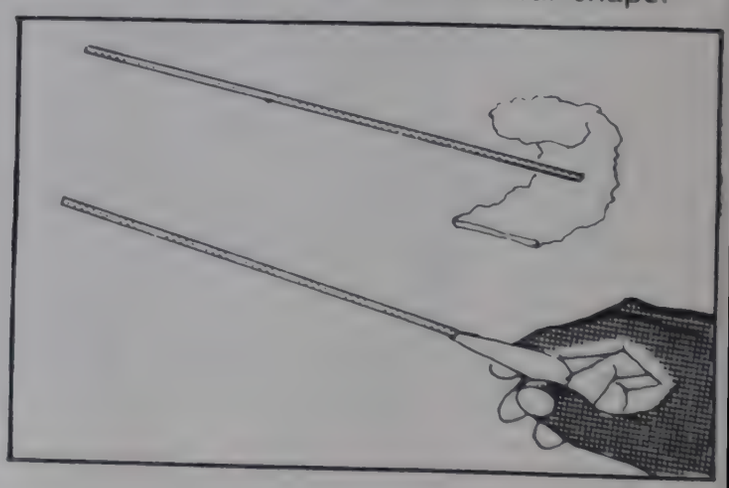
i. Prepare some thin sticks of wood (or aluminium wire), 18 cm long and 2 mm in diameter.

Prepare strips of cotton wool, 6 cm long by 3 cm wide and as thin as possible.

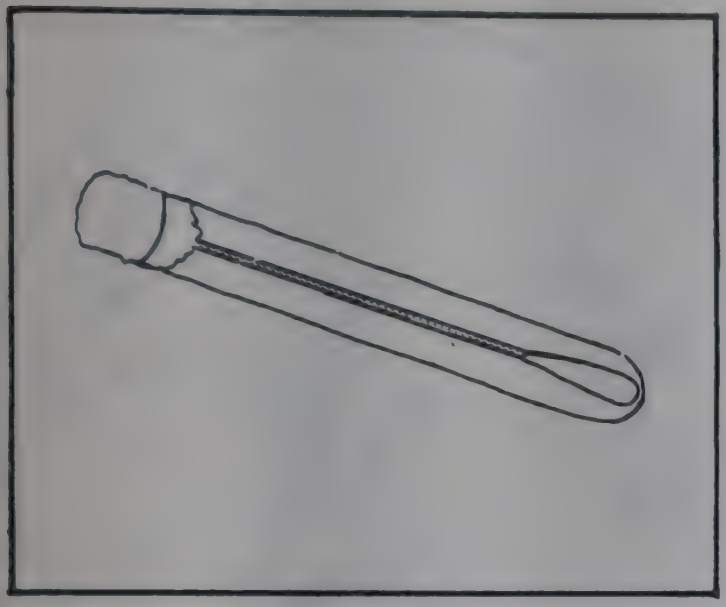


ii. Roll the cotton wool round one end of the stick. If metal wire is used, flatten the end first.

iii. Mould the swab in a conical shape.



iv. Place in a thick Pyrex tube. Plug with non-absorbent cotton wool. Sterilize by autoclaving.



2. Collection of Specimen

Ideally this should be done by the physician or nurse, but the laboratory technician may be called upon to take the specimen.

If the smear is to be inoculated on a culture medium or despatched in a transport medium, take two swabs:

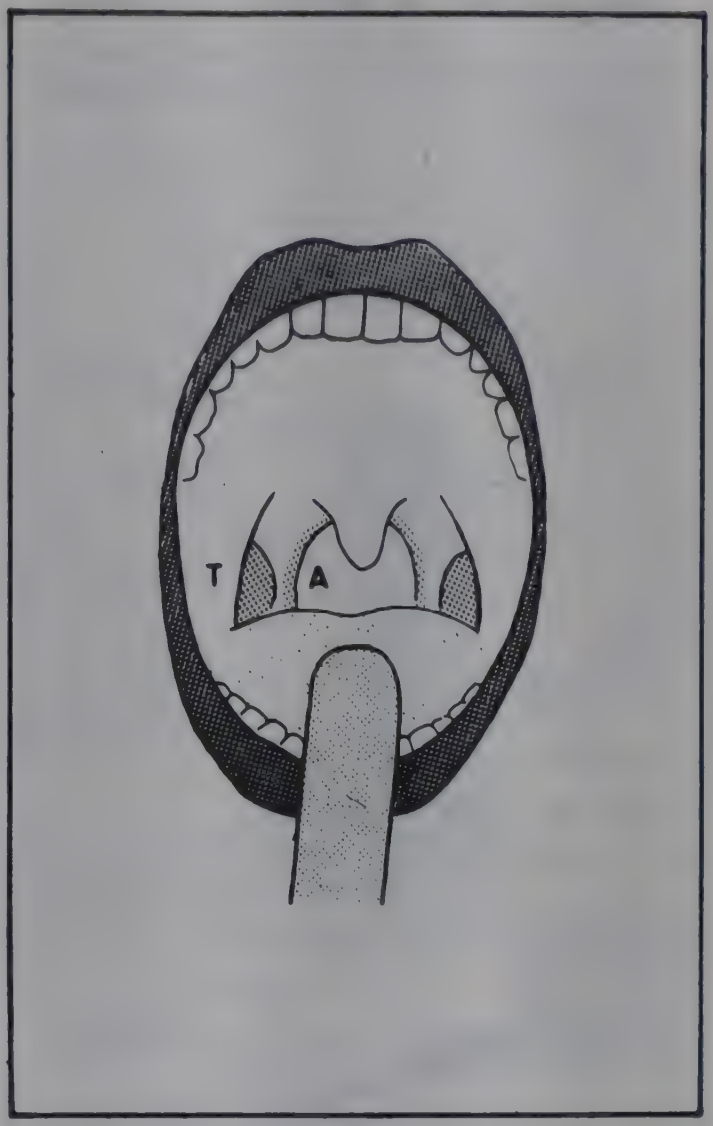
- the first for the medium
- the second for preparing smears for direct examination.

If diphtheria is suspected, a swab should be placed on Loeffler culture medium (obtainable from the State reference laboratory) and sent to a specialized laboratory.

(1) The patient should sit facing the light (or an electric torch can be used).

(2) Tell the patient to open his mouth without putting out his tongue, and to say 'Ahhhhh. .'.

(3) While he is saying 'Ahhhh. .', press the outer ⅔ of the tongue down with the tongue depressor using the left hand. The tonsils (T) and the back of the throat framed by the arches (A) should be visible.



(4) Introduce the swab with your right hand. Do not touch the tongue, which is coated with organisms.

(5) Locate the infected (inflamed) part of the throat.

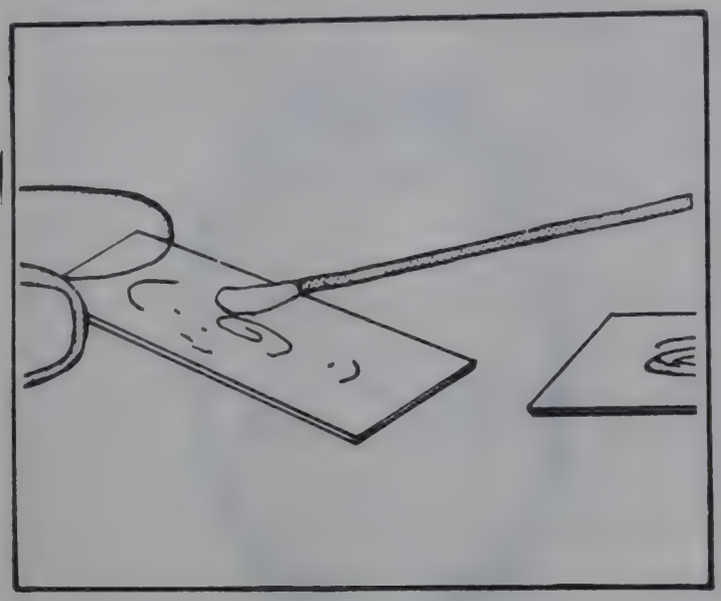
It will be very red or white depending on the case. Usually the infection is located in the tonsils or fauces.

(6) Rub the swab firmly against the inflamed part, turning it round, and collect membranes, if present.

(7) If nothing abnormal is seen, swab the tonsils, the fauces and the back of the soft palate.

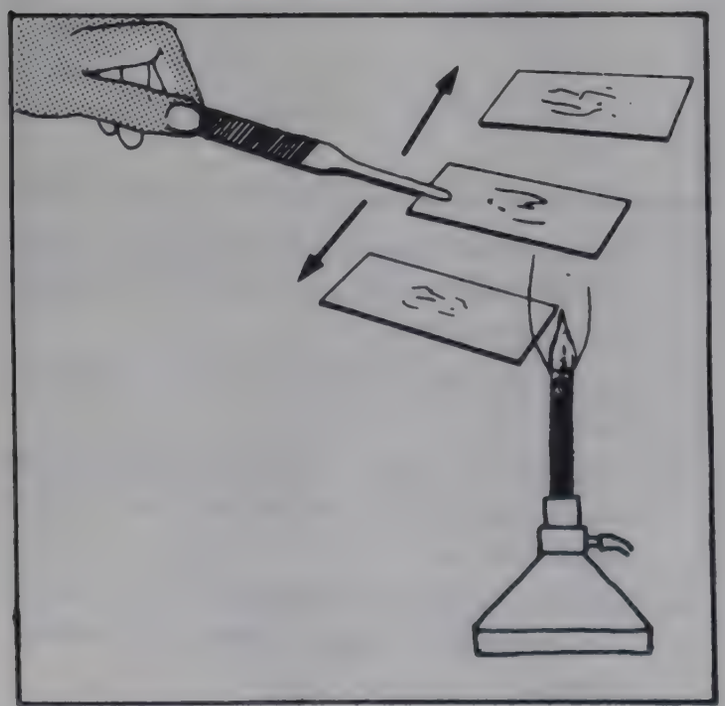
3. Preparation of Smear

(1) Streak 2 or 3 slides with the swab, using a rolling movement to make sufficiently thick broad smears.



(2) Dry the smear in the air.

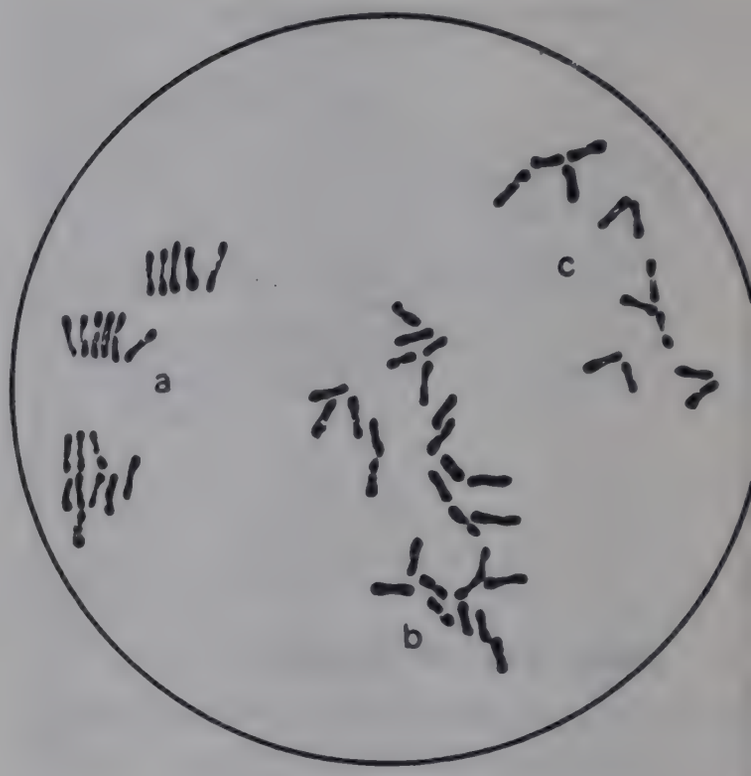
(3) Pass the slide 3 times through the flame of a Bunsen burner with the smear uppermost.



Allow to cool.

(4) Stain the smear by the Gram method (See Appendix 7.1).

4. Description of Diphtheria Bacilli



Narrow Gram positive rods, straight or slightly curved and often enlarged at one end or both ends. They are arranged:

- in rows (a)
- or scattered (b)
- or in V formations (c).

5. Reporting the Result

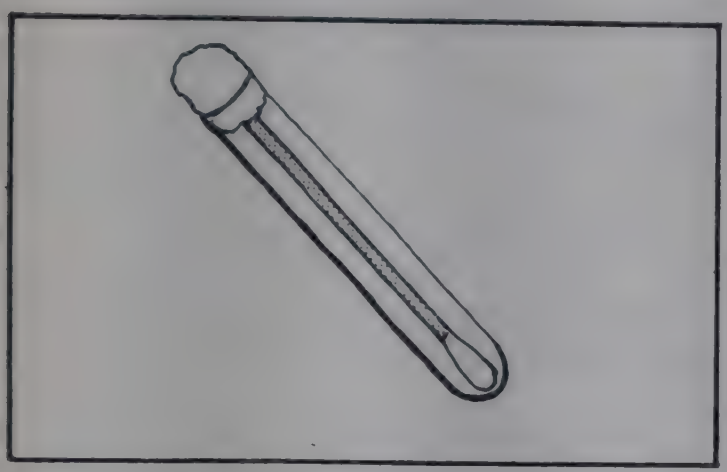
Note the various elements found in the smear, e.g. leucocytes, red blood cells, epithelial cells, gram positive and/or gram negative cocci or bacilli.

When reporting the presence of diphtheria-like bacilli in a throat swab, state that it shows the presence of Gram positive bacilli resembling Corynebacterium diphtheriae. The reason for this is that diphtheria bacilli can only be identified with certainty following culture.

6. Despatch of Throat Specimens
(to a bacteriology laboratory for culture)

(1) Despatch of swab alone

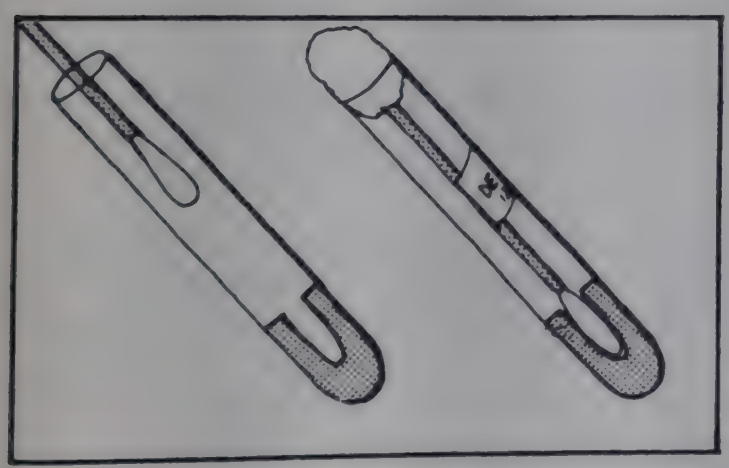
As soon as the specimen has been collected, replace the swab in its sterile tube and send it as it is to the bacteriology laboratory.



Preservation time: 4 hours at the maximum.

(2) Despatch for the detection of diphtheria bacilli using Loeffler medium in a special tube for swabs

Insert the swab immediately after collection of the specimen in the cylindrical space in the centre of the medium.



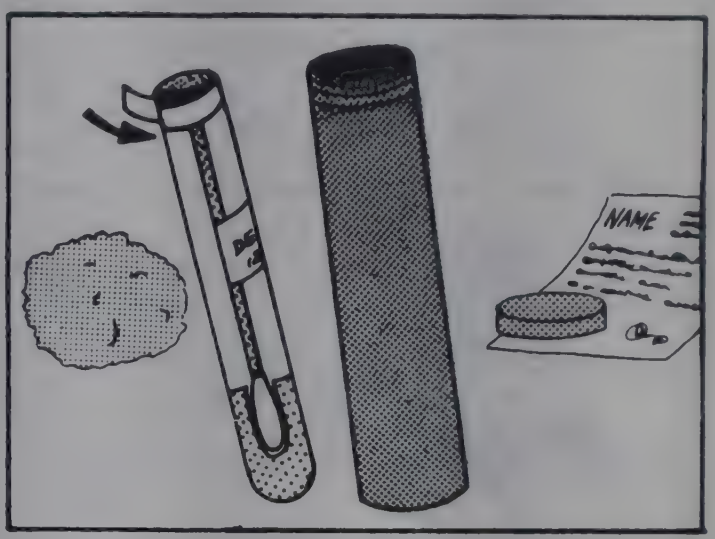
Send the same day.
Maximum transport time: 24 hours.

(3) Packing

Always observe the regulations in force in your State.

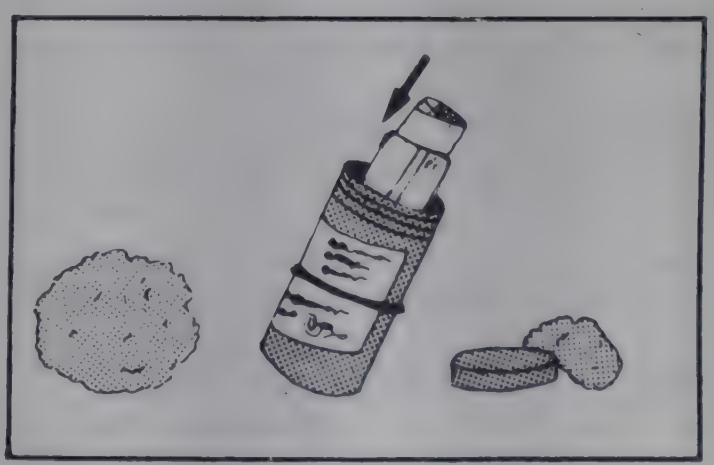
Double pack the specimens.

i. After placing the specimen in the sterile tube, seal the tube hermetically (fixing the stopper with sticking plaster).



ii. Check that the tube is labelled with the patient's name.

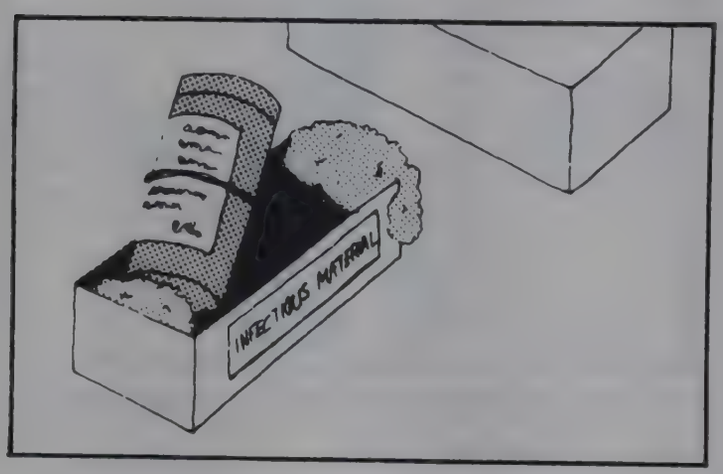
iii. Place the sealed tube in an aluminium container with a screw cap. Wedge it in the container with absorbent cotton wool.



iv. Wrap the request form around the metal container and fix with a rubber band.

The request form should show:
- the patient's NAME (written in capital letters), first name, sex and age
- the nature of the specimen
- the examinations requred (with the physician's diagnosis, where appropriate).

v. Place the metal container in a strong cardboard or wooden box for despatch. Wedge it tightly with non-absorbent cotton wool.



vi. Mark on the label on the outside of the box:

URGENT
FRAGILE
INFECTIOUS MATERIAL

CHAPTER 14

Examination of Water Sample

The requirements for wholesome drinking water are as follows:

- It should not have any objectionable taste, odour or colour.
- It should not contain an undue amount of solid particles (turbidity).
- It should be free from nitrites which indicate active decomposition of organic matter.
- It should not contain toxic substances, such as lead, arsenic or mercury.
- It should not have a high fluoride content.
- It should not contain pathogenic organisms.
- It should not contain bacteria such as Escherichia coli, which indirectly indicate that faecal pollution has occurred.

Various tests must be carried out to determine whether water is safe for humans to drink. These tests are designed:

- to determine the physical quality of the water
- to check whether the amount of various chemical substances is within the normal limits
- to detect the presence of chemical pollutants
- to identify and count the number of organisms that contaminate the water
- to determine the free residual chlorine.

These tests are usually performed in specialized Public Health Laboratories. However, as a technician working in the PHC laboratory, you must know how to collect and despatch water samples for testing at the Public Health Laboratory. You can also carry out certain simple tests in the PHC laboratory itself. You should also be aware of the remedial measures to be taken based on the results of water testing.

1. Collection and Despatch of Water Sample

The value of any laboratory analysis of water depends on the method of sampling. Failure to observe proper precautions in collecting a representative sample may result in an analysis which is of little or no value since it may unnecessarily condemn a good water supply; or, what is worse, may certify a bad water supply as satisfactory.

(1) Collection for microbiological examination

In order that microbiological examination of the water sample can be effectively carried out, it is important that the water sampling should fulfil the following requirements:

i. Sampling should be properly planned and carried out with sufficient frequency. The sampling points should be representative of the water sources used by the community. They should include those points where possible contamination could occur. The sampling points should be located in proportion to the population served. This is explained further below.

ii. Samples should be collected, stored and despatched in suitable sterilized glass bottles with properly fitting stoppers or caps. The bottles should be adequately sterilized as explained later.

iii. The volume of water collected should be

large enough for accurate analysis. The sample bottle should hold at least 200 ml of water.

iv. If the sample for microbiological analysis contains any residual chlorine, the latter will continue to act on any bacteria present after sampling. This will give false results. To avoid this, sodium thiosulphate must be added to the sample bottle. This will destroy any residual chlorine present but will not affect the microorganisms in the sample.

v. Great care should be taken during sampling to prevent contamination of the sample being collected as explained later.

vi. In order to prevent any significant change in the composition of a collected sample prior to its analysis, it is important to ensure that it is collected properly and then despatched as rapidly as possible.

vii. The sample details should be adequately described and the sample bottle properly labelled to avoid errors.

Materials

- 250 ml Pyrex glass bottle with ground glass stopper or screw cap, washed and rinsed with distilled water
- Clean cloth
- Brown wrapping paper
- String
- 10% solution of sodium thiosulphate (Reagent No. 26)
- Cotton wool swab
- 70% ethanol
- Matches

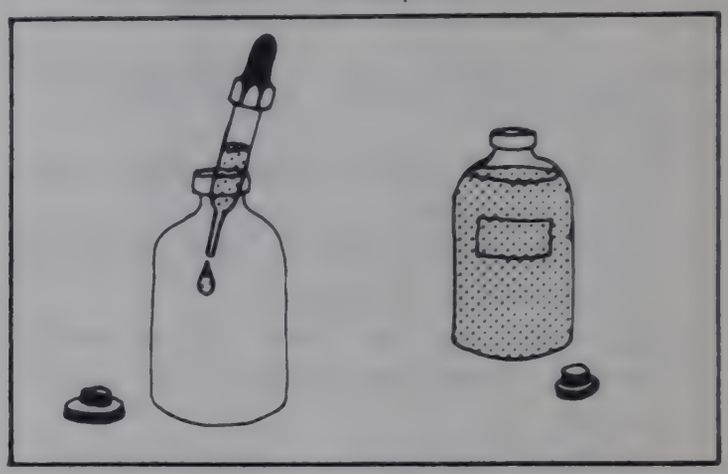
Method

The water sample may be taken from:

- a tap or the outlet from a fixed hand pump
- a dugwell
- a water course or reservoir, such as a lake, river or tank.

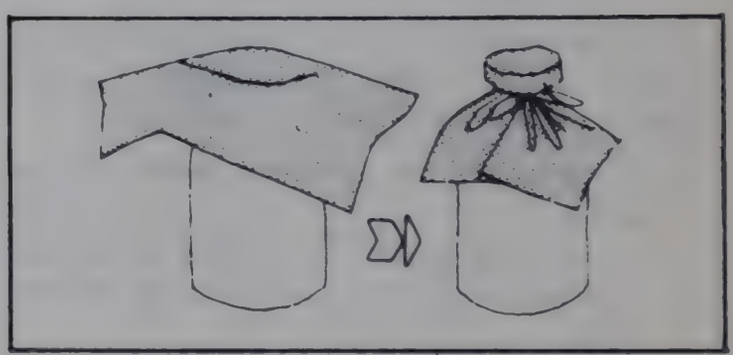
Preparations

i. Place in 250 ml sampling bottle 0.2 ml of 10% sodium thiosulphate solution.



Note: Sodium thiosulphate is necessary for dechlorination of chlorinated water samples.

ii. Replace the ground glass stopper or screw cap. Cover the stopper with a piece of brown wrapping paper and tie it on firmly with string.



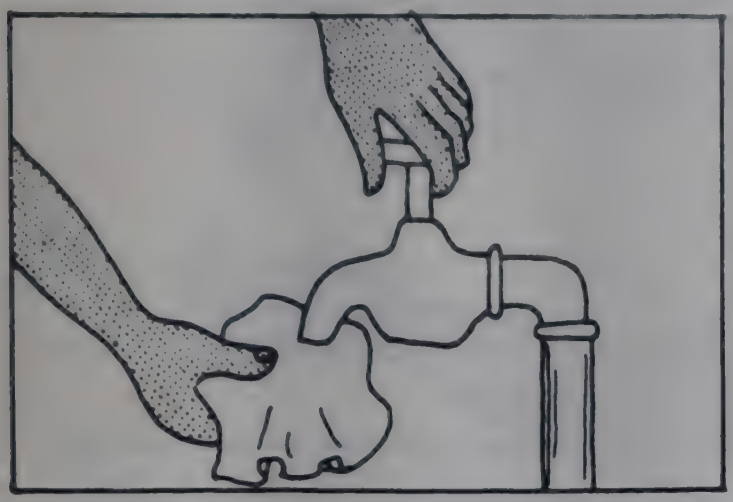
iii. Sterilize in the autoclave at 1 kg/cm^2 pressure for 15 minutes;

or in a pressure cooker.

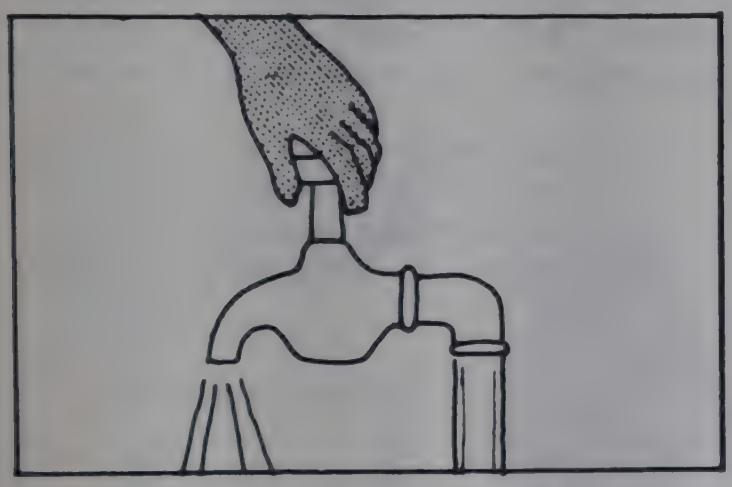
Do not open the bottle till the time of filling.

(a) *Sampling from a tap or pump outlet*

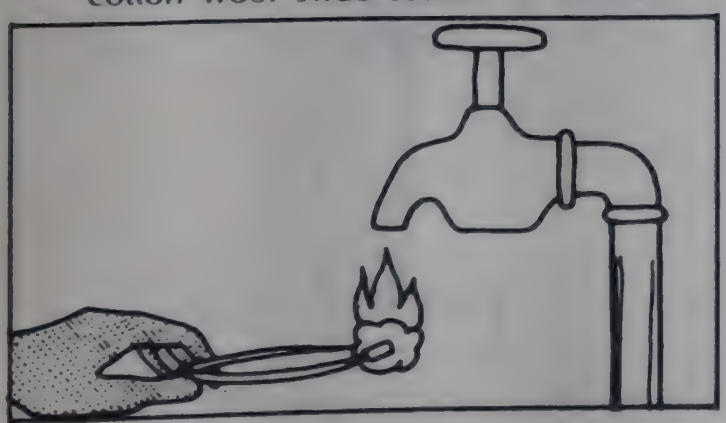
i. *Tap cleaning*: Remove from the tap any attachments that may cause splashing. Using a clean cloth, cleanse the outlet in order to remove any dirt.



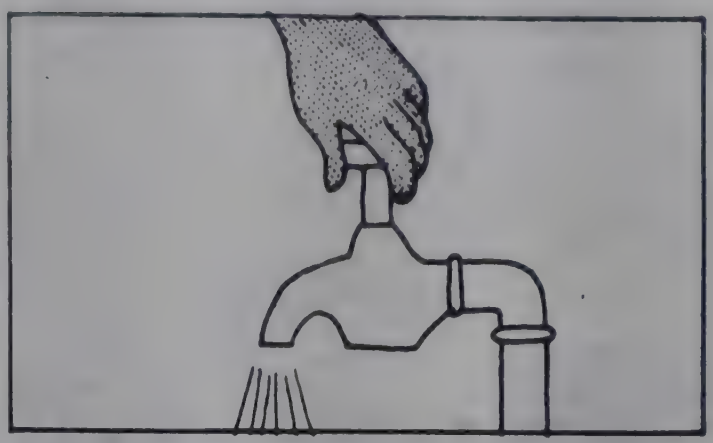
ii. *Opening the tap*: Turn on the tap at maximum flow rate and let the water flow for 1–2 minutes.



iii. *Sterilizing the tap*: Sterilize the tap for a minute with the flame from an ignited cotton wool swab soaked in alcohol.

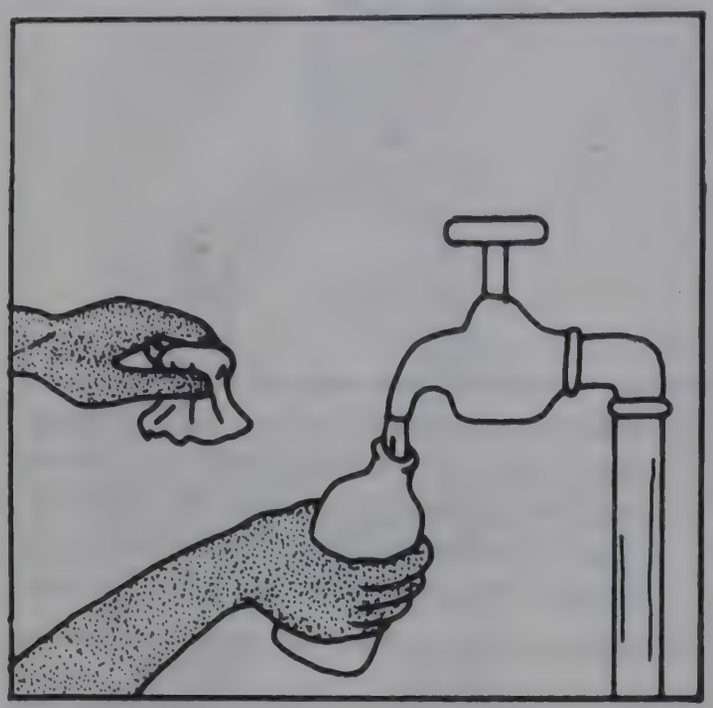


iv. *Opening the tap prior to sampling*: Carefully turn on the tap and allow the water to flow for 1–2 minutes at a medium flow rate.

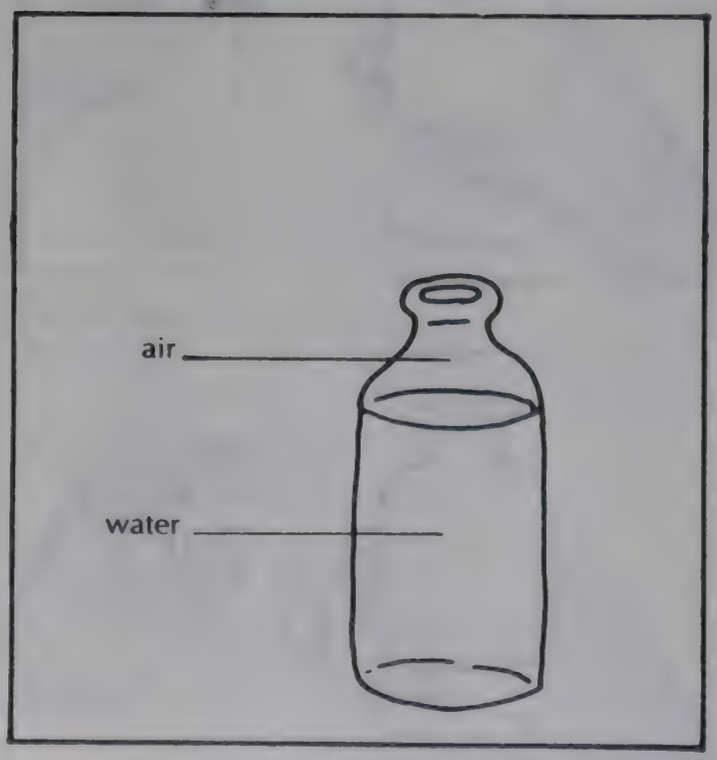


v. *Opening sterilized bottles*: Take a sterilized 250 ml sample bottle containing sodium thiosulphate. Untie the string fixing the protective brown paper cover and remove the stopper or unscrew the cap.

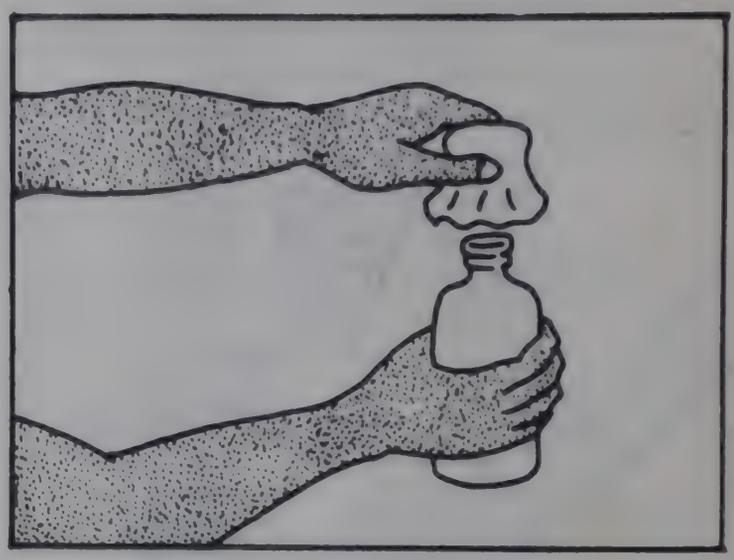
vi. *Filling the bottles*: While holding the cap and protective cover face-downwards (so as to prevent entry of dust that might carry microorganisms), hold the bottle immediately under the water jet and fill it.



Leave a small air space to facilitate shaking at the time of inoculation prior to analysis.



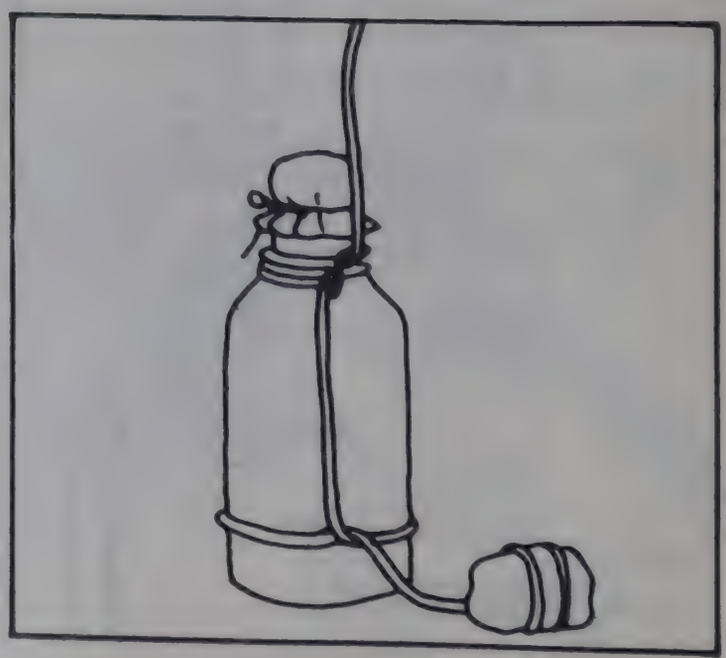
vii. *Stoppering bottles*: Replace the stopper in the bottle or screw on the cap. Fix the brown paper protective cap in place with the string.

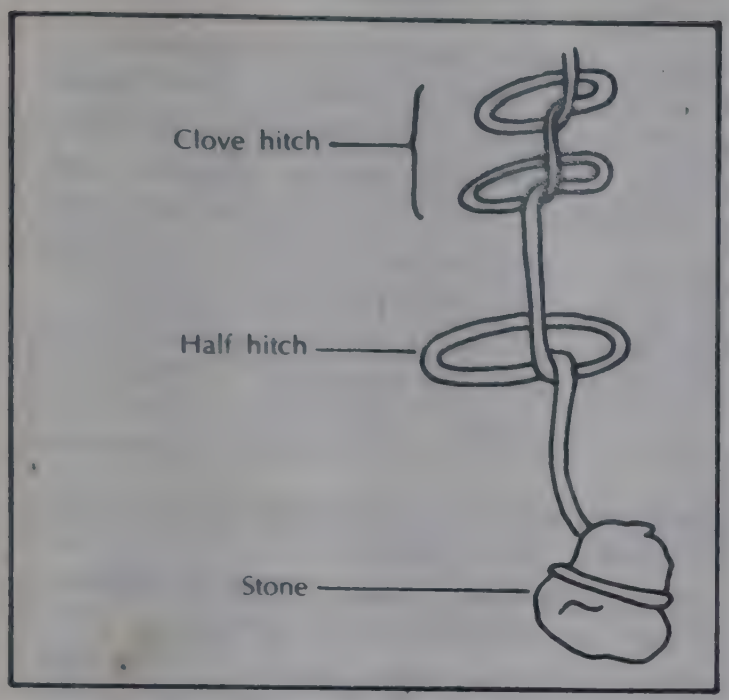


(b) *Sampling from a dugwell*

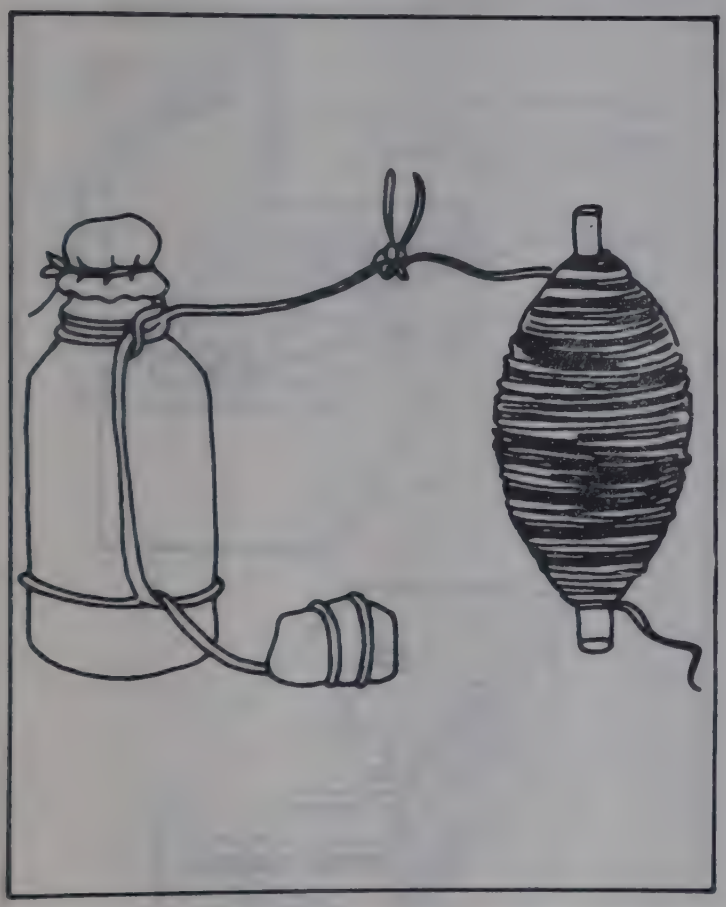
Note: The sample should be collected only after the well has been pumped to waste for 4 to 5 minutes. This will ensure that the sample is representative of the ground water in the well.

i. *Initial preparation*: Take a sterilized 250 ml sample bottle containing sodium thiosulphate. At the well, attach a suitable sized stone with a string to the sampling bottle.



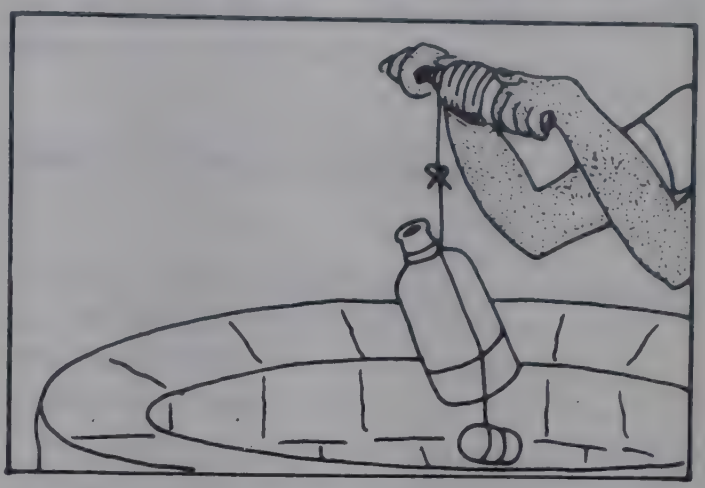


ii. *Preparations for lowering bottle*: Take a 20 metre length of clean string rolled around a stick and tie it on to the bottle string.

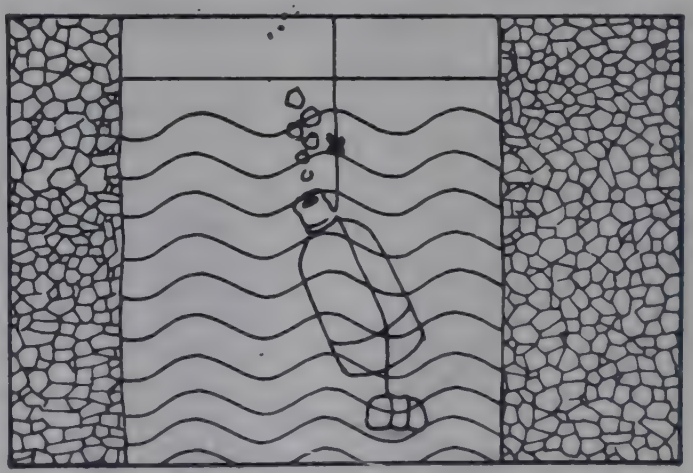


iii. *Opening sterilized bottles*: Untie the string fixing the protective brown paper cover and remove the stopper or unscrew the cap.

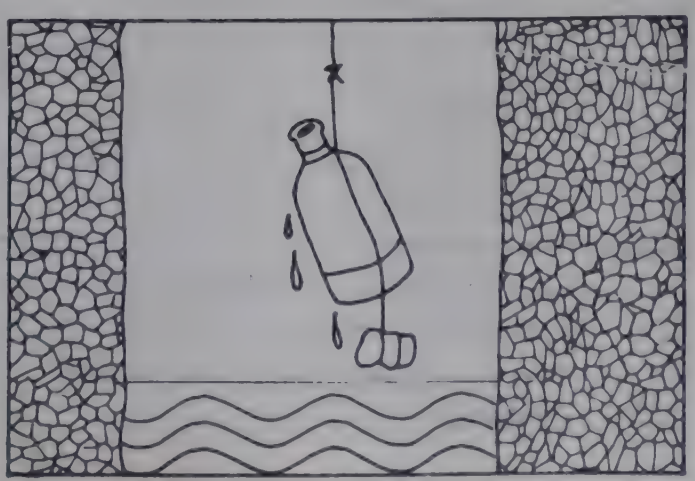
iv. *Bottle lowering:* Lower the bottle, weighted down by the stone, into the well, by unwinding the string slowly. Do not allow the bottle to touch the sides of the well.



v. *Bottle filling*: Immerse the bottle completely in the water down to the bottom of the well.



vi. *Bottle raising*: Once the bottle is judged to be filled, rewind the string round the stick to bring up the bottle full of water.



Discard some water if the bottle is completely full, to provide an air space.

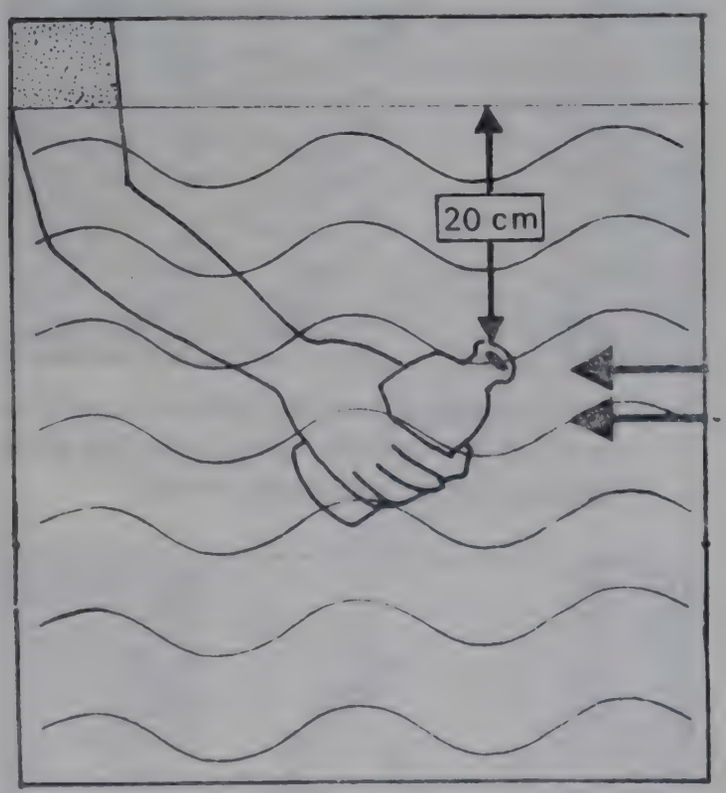
vii. *Stoppering bottles*: Replace the stopper in the bottle or screw on the cap. Fix the brown paper protective cap in place with the string.

(c) Sampling from a water course or reservoir

(lake, stream, river, or tank)

i. *Opening sterilized bottles*: Take a sterilized 250 ml sample bottle *without* sodium thiosulphate. Untie the string fixing the protective brown paper cover and remove the stopper or unscrew the cap.

ii. *Filling the bottles*: Holding the bottle by the lower part, submerge it to a depth of about 20 cm water, with the mouth facing slightly upwards. If there is a current of water, the bottle mouth should face towards the current.



iii. *Stoppering bottles*: Replace the stopper in the bottle or screw on the cap. Fix the protective brown paper cap in place with the string.

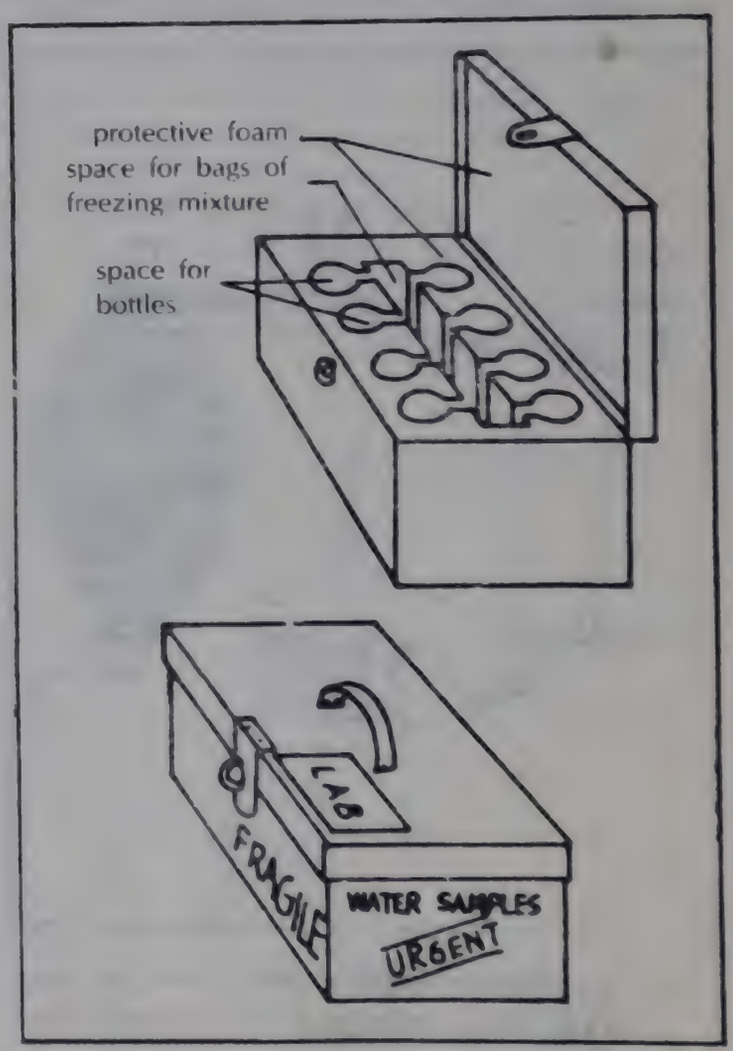
(d) Packing and despatch

Bottles should be transported or despatched in a strong box to prevent breakage. There should be sufficient space in the box to include bags of freezing mixture for keeping samples cool. Boxes holding six to twelve bottles are ideal. The outer cover can be of wood or metal and should bear the clearly written inscription,

FRAGILE WATER SAMPLES URGENT THIS SIDE UP

as well as the address of the laboratory to which the bottles are to be sent.

It is convenient to have a reversible plate on the box lid, with the name and address of the person collecting water samples on one side, and that of the water analysis laboratory on the other. The lid should have a handle to help ensure that the box is carried the right way up.



Samples should be sent to the water analysis laboratory as quickly as possible, ideally within 6 hours of collection but never more than 24 hours later. The ideal temperature for sample storage is 4 – 10 °C.

Often there will not be a vehicle for the transportation of sample bottles, and consequently public transport has to be used. This means that considerable attention must be paid to timetables and routes.

(2) Collection for physical and chemical analysis

Collect the sample in a container of Pyrex glass or polythene.

Before use clean the sample bottle carefully. Glass bottles should be rinsed with chromic acid cleaning mixture*. Rinse thoroughly with tap water and then with distilled water.

* Add 1 litre of concentrated sulphuric acid *slowly with stirring* to 35 ml saturated sodium dichromate solution.

About 2.5 litres of water are required for analysis.

Before filling, rinse out the sample bottle twice or thrice with the water to be collected.

The sample should be examined within 72 hours of collection.

2. Frequency of Sampling

Examination of drinking water should be both frequent and regular. The frequency of sampling will depend on:

- the quality of the source
- the treatment the water receives
- the risks of contamination
- the previous history of the supply
- the size of the population served
- whether or not the source of water supply is a new one.

The quality of water will vary with the season and with the proximity of sources of pollution. The frequency should, therefore, be decided upon by the concerned health authorities depending on the local circumstances and the results of sanitary surveys.

A new source will need more frequent sampling so that variations in quality can be observed under a variety of weather conditions.

The *minimum* sampling frequencies are suggested in the table on p. 202. The sampling should be increased at times of epidemics, flooding, or following interruption of supplies or repair work. Take the advice of the MO PHC as to how often the samples should be taken.

Frequency of sampling

Type of supply	*Residual chlorine test*	*Sample collection for despatch to Public Health Laboratory*	*Remarks*
1. Open dugwells which are chlorinated	At least once a month.*	Once initially. Thereafter if change in environmental conditions, outbreaks of waterborne diseases, or undue increase in waterborne diseases is reported.	Open wells are generally unsafe and steps should be taken for covering such wells to protect them from inflow of pollution.
2. Covered dugwells with hand pumps and deep tube wells with hand pumps	–	-do-	Assist the Health Worker and Health Guide in inspecting the water source to detect actual or potential contamination by humans or animals.
3. Piped supplies from underground sources (including protected springs)	At least once a month where supplies are chlorinated.*	-do-	-do-
4. Piped supplies from rivers, canals, lakes, reservoirs, open tanks or other surface sources	At least once a day.*	*Population* / *Minimum No. of samples* Less than 5,000 — 1 sample per month 5,000 – 100,000 — 1 sample per 5,000 population per month More than 100,000 — 1 sample per 10,000 population per month	More frequent sampling may be carried out in case of change in environmental conditions, outbreaks of waterborne diseases or undue increase in waterborne diseases.

* This should be routinely done by the Health Guide or Health Worker Male and occasionally checked by the Laboratory Technician or Health Assistant Male.

3. Physical Examination of Water

You will not be able to carry out a quantitative analysis of water. However, you can observe the water sample for the following physical characteristics:

(1) Colour

Pour the water into a colourless glass cylinder about 60 cm high placed on a white plate. The longer the cylinder, the better is the colour noticed.

Pure water is colourless. When viewed in bulk it has a slight greenish tinge.

A yellow or brown colour may be due to:

- contamination with animal organic matter, e.g. sewage
- vegetable matter
- salts of iron.

(2) Turbidity

Shake a small quantity of water in a test tube. Note whether it looks:

- clear (normal)
- hazy or turbid (this may be due to clay or silt, or minute particles of organic matter).

(3) Lustre or brilliance

Note whether the water is:

- clear and sparkling (contains carbon dioxide and air)
- dull or slimy (due to suspended matter)

(4) Taste and odour

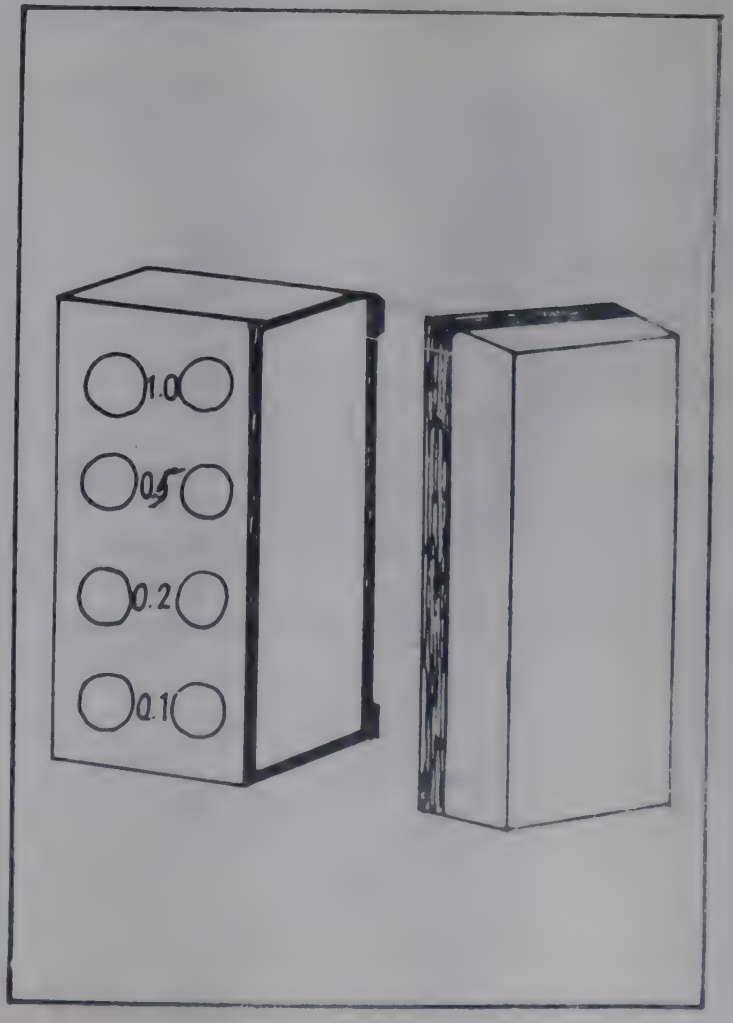
- Distilled water has no taste or odour and is insipid, whereas water in its natural form is rendered palatable by the presence of calcium and magnesium ions.
- In most cases gases and minerals impart a taste to water.
- Presence of algae in the water gives it a disagreeable and nauseous taste.
- Offensive odour may be cuased by:
  * stagnation of the water
  * presence of dead animals in the water
  * hydrogen sulphide
- Chlorination of water gives it an iodoform taste.

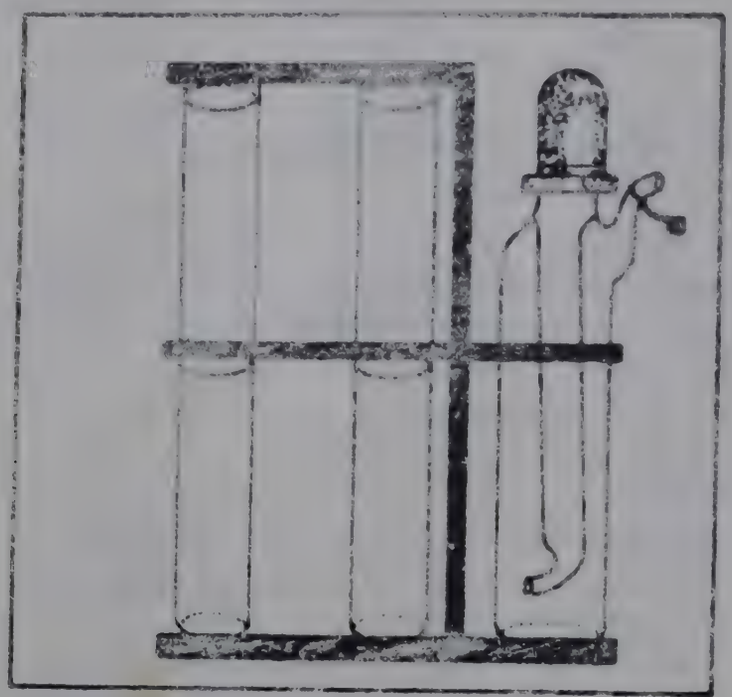
4. Free Residual Chlorine (Orthotolidine) Test

Measurement of the chlorine residual is quick and easy to perform. It gives an indication of the presence of contamination in chlorinated water sources. It is desirable to maintain a free residual chlorine of 0.2 to 0.5 mg/litre (also expressed as parts per million *or* p.p.m.) in the distribution system in order to reduce the risk of microbial regrowth. It it falls below this level, it indicates that there has been contamination following chlorination.

It is useful for you to know how to carry out this test, so that you can assist the field staff in carrying out effective chlorination.

Materials





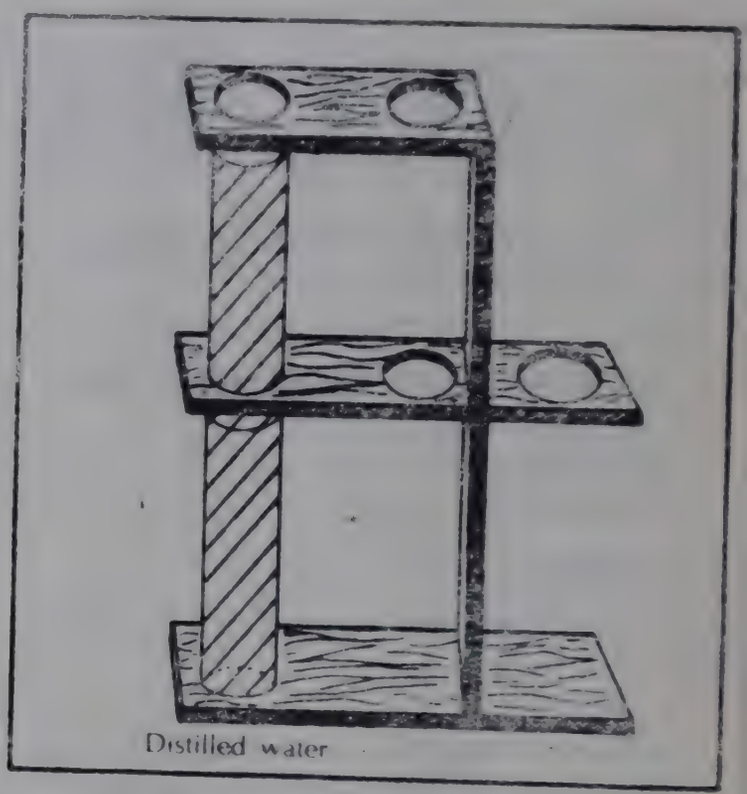
– Orthotolidine Set (Chloroscope) consisting of:
  * chloroscope box with 4 standard discs (0.1, 0.2, 0.5 & 1.0 p.p.m.) and ground glass plate
  * 2 sample tubes each with two circular markings
  * orthotolidine reagent dropper
  * sample holder
  * dropping pipette
  Orthotolidine reagent (Reagent No. 22)
  Distilled water

Method

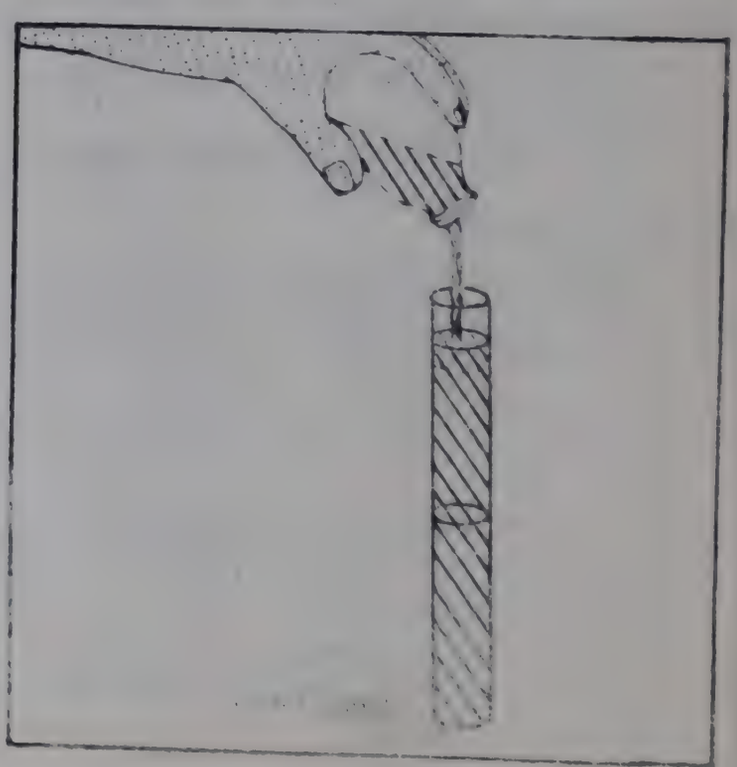
i Open the tygon cap and the side cap of the reagent dropper.
Fill the dropper to ¼ of its volume with OT Reagent

Replace the tygon cap and the side cap. Place the dropper in the sample holder.

ii. Fill the left hand tube with distilled water up to the upper mark. Insert it in the sample holder.

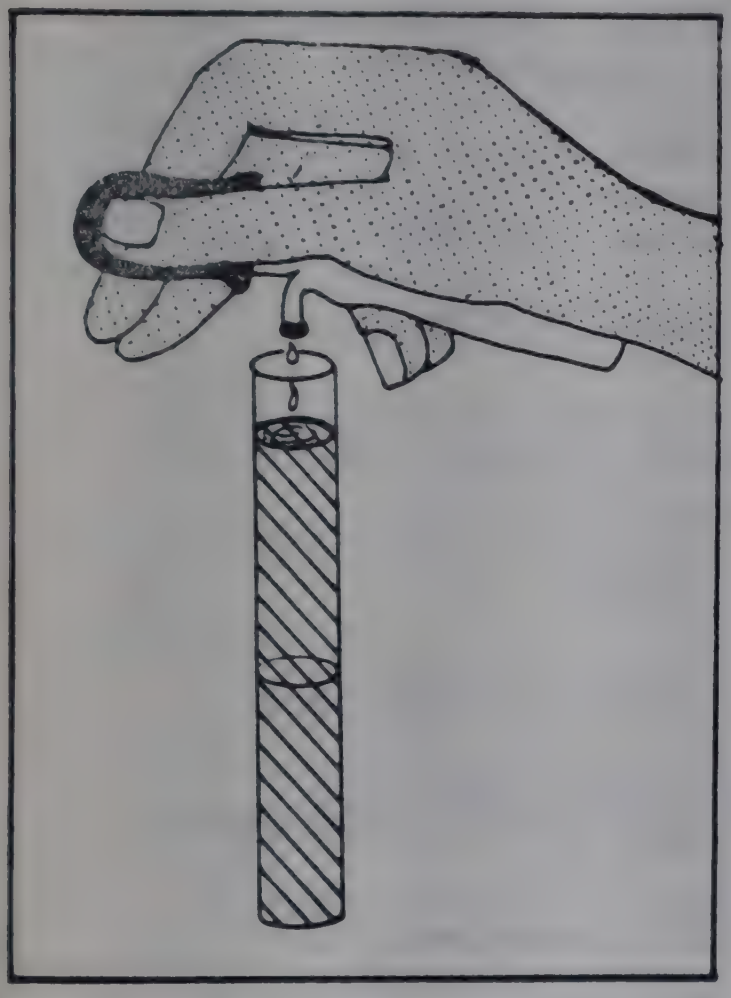


iii Fill the right hand tube up to the upper mark with the water to be tested.



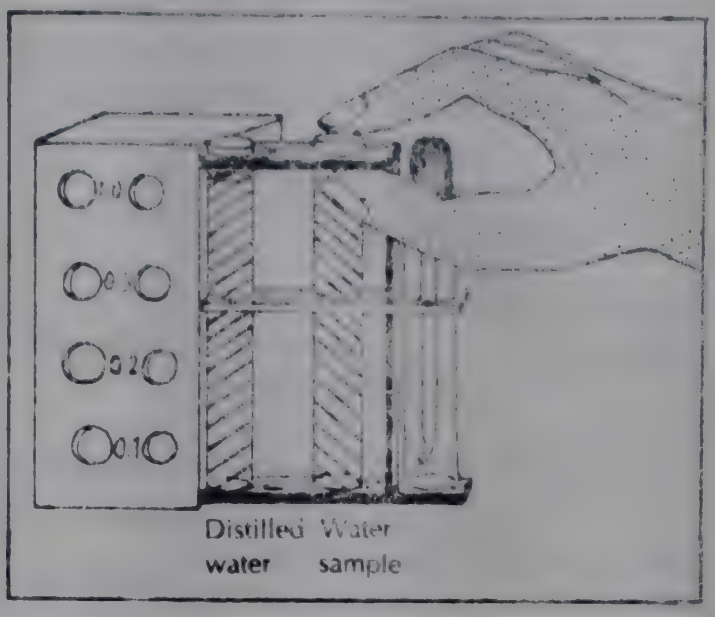
Note: In case of a concentrated sample, the tube may be filled up to the mid-way mark only and then diluted with distilled water.

iv. Remove the tygon cap of the dropper bottle and, holding it above the sample tube, press the side cap to allow 4 drops of OT Reagent to be added to the water in the sample tube.

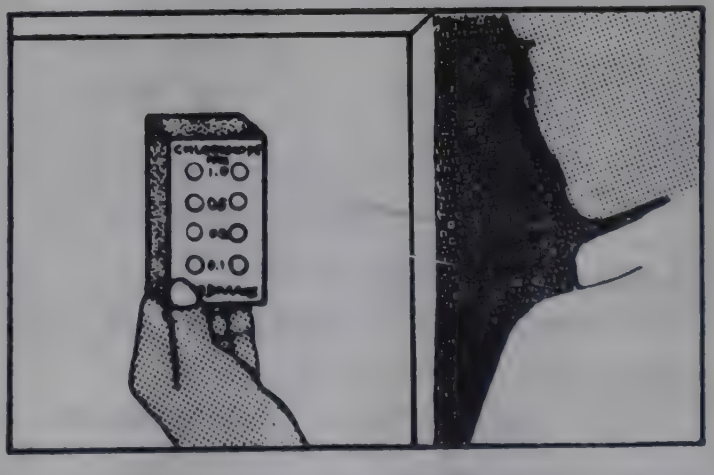


v. Shake the sample tube and replace it in the sample holder.

vi. Insert the sample holder in the chloroscope box and insert the side cover.



vii. Hold the chloroscope against the daylight, keeping the ground glass side away from the eye.



viii. Match the developed colour of the test water with one of the four standard discs. The colour will vary from light yellow to deep orange, depending on the quantity of residual chlorine present in the sample. A deep orange colour indicates that the residual chlorine is too high.
Note the reading.

Results:

0.1 p.p.m.	: water is insufficiently chlorinated
0.2 p.p.m.	: suitable for use
0.5 p.p.m.	: suitable for use during rainy season or epidemics
1.0 p.p.m.	: too much chlorine present

Note: In order to obtain accurate results it is essential that the reading should be carried out *within 30 seconds* of adding the orthotolidine reagent.
In case of absence of free residual chlorine the water supply authorities should be contacted for carrying out remedial measures.

5. Request Form and Records

In order to ensure that every sample is clearly and adequately described, it should be accompanied by a detailed request form. This form should contain all the information related to where and when the sample was collected, as well as a description of the source, and the name of the person who collected and despatched the sample.

An example of a request form is given below:

Request for water analysis

PHC:
Water collected at: (locality) (exact spot)
Date of collection: . Time of collection:
Source of supply: Tap ☐ Standpipe with pump ☐ Well ☐ (depth:)
Spring ☐ River ☐ Lake ☐ Reservoir ☐ Pond ☐ Other (specify):
Is the water used for drinking?
Are there latrines in the vicinity? Yes ☐ No ☐ . . . . . metres away
Remarks*:
Sample collected by: Signature:
Sample received by: Date: Time: Signature:

*State whether there has been any recent rainfall, floods or drought. State if there has been any recent outbreak of a water-borne disease in the area.

Keep a record of the collection of water samples and examinations (if any) carried out by you on the water samples (See Appendix 5.2H).

Fill in the report of the examinations carried out by you on the water sample (See Appendix 5.3 H) and submit it to the Medical Officer of the PHC.

6. Remedial and Preventive Measures

The purpose of water quality surveillance is to control its quality and thus protect the consumer. It is, therefore, not enough merely to carry out water sampling and analysis. It is important that, based on the results of these tests, remedial measures should be taken:

- to correct or eliminate any problems identified.
- to take the necessary emergency precautions to prevent water-borne epidemics.

Such remedial measures include the following:

Immediate measures

- Cleaning and disinfection ('shock chlorination' with a heavy dose of chlorine or bleaching powder) of wells.
- Identification of alternative safe sources of water.
- Education of the community to chlorinate their wells and to boil water before drinking.
- Confirmation by microbiological analysis and/or residual chlorine testing that remedial measures have been effectively carried out.

Long-term measures

- Selection of safe, adequate sources of water.
- Repair and maintenance of wells and pumps.
- Conversion of open dugwells to protected covered wells with handpumps.
- Removal of any source of pollution of the water source.
- Protection of the water source and its catchment.
- Regular chlorination of wells.
- Constant vigilance and periodic sanitary checks.
- Continuing education of the community and involvement of the community in ensuring a safe water supply.

As a member of the health team at the PHC, you are also responsible for encouraging the people in your area to strive towards a safe water supply. Some of the activities in which you can assist the Health Workers and Health Guides are as follows:

- Inspecting the water sources to detect actual or potential contamination of water by humans or animals.
- Reporting the findings of inspection to the Village Health Committee and the MO PHC.
- Devising and implementing with the help of the community methods for protecting the water source from contamination.
- Advising the community about how to prevent contamination of the water source.
- Taking samples of water periodically for transport to and analysis at the nearest Public Health Laboratory.
- If the water source is contaminated, carrying out residual chlorine tests.
- Informing the community of the results of water analysis, the implications of these results with respect to health, and the remedial measures which require to be taken to keep the water clean and safe.